UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: August 30, 2017

SUBJECT: **Benzovindiflupyr DERs**

PC Code: 122305
Decision No.: 525092
Petition No.: 6F8542
Risk Assessment Type: N/A
TXR No.: 0057632
MRID No.: See MRID Table

DP Barcode: D441992
Registration No.: N/A
Regulatory Action: N/A
Case No.: N/A
CAS No.: 1072957-71-1
40 CFR: N/A

FROM: John Liccione; Ph.D., Toxicologist
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THROUGH: Mike Metzger, Branch Chief
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TO: Fatima Sow, Risk Manager Reviewer
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I. CONCLUSIONS

The toxicology DERs supporting the registration of benzovindiflupry have been finalized by HED.

II. ACTION REQUESTED

Review toxicology DERs submitted by PMRA as part of the joint registration of benzovindiflupry in the United States and Canada.

III. BACKGROUND

PMRA was the primary reviewer for toxicity studies on benzovindiflupry. The Agency has provided a secondary review of the DERs.

Guideline	**MRID#**	**Study Title**
870.5100	47473401	NOA449109 – *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay
870.5100	47746852	CSCD465008 – *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay
870.5375	47923610	CSAA798670 – Chromosome Aberration Test in in *Vitro.*
870.5300	47923611	CSAA798670 – Cell Mutation Assay at the Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y Cells
870.7485	48604420	SYN545192 - Investigation of the Nature and Identity of Radiolabelled Metabolites Present in Plasma, Urine, Faeces and Bile Collected from Rats Following Oral Administration of [^{14}C]-SYN545192
870.7485	48604421	SYN545192- An Investigation of the Tissue Distribution (QWBA) of Total Radioactivity in the Rat Following Oral Administration of Pyrazole or Phenyl Labelled [^{14}C]-SYN545192
870.7485	48604422	SYN545192 – The Biliary Elimination of Total Radioactivity in the Rat Following Single Oral Administration of [Pyrazole-^{14}C]-SYN545192
870.7485	48604423	SYN545192- The Tissue Depletion of [Pyrazole-^{14}C]-SYN545192 in the Rat Following Single Oral Administration.

870.7485	48604424	SYN545192- The Tissue Distribution and Elimination of [Pyrazole-^{14}C]-SYN545192 in the Rat Following Repeated Daily Oral Administration
870.7485	48604425	SYN545192 – The Excretion and Tissue Distribution of [^{14}C]-SYN545192 in the Rat Following Single Oral Administration
870.7485	48604426	SYN545192 – The Pharmacokinetics of [Pyrazole-^{14}C]-SYN545192 the Rat following a Single Oral Administration.
870.3050	48604433	Twenty-Eight Day Repeated Oral (Dietary) Toxicity Study in the Rat;
870.3050	48604435	SYN545192 - 28 Day Mouse Dietary Toxicity Study
870.3100	48604436	SYN545192 – 90 Day Dietary Study in Rats.
870.3100	48604437	SYN545192 – 90 Day Dietary Study in mice
870.3150	48604439	SYN545192 – 13-Week Oral (Capsule) Toxicity Study in the Beagle Dog
870.3150	48604440	SYN545192 – 13-Week Oral (Capsule) Toxicity Study in the Beagle Dog.
870.3200	48604441	SYN545192 – 28-Day Dermal Toxicity (Semi-Occlusive) study in the Wistar Rat.
870.5100	48604442	SYN545192 tech. – *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay
870.5375	48604443	SYN545192-Chromosome Aberration Test in Human Lymphocytes *in Vitro*
870.5300	48604444	YN545192 – Cell Mutation Assay at the Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y Cells
870.5395	48604445	SYN545192 – Micronucleus Test in Bone Marrow Cells of Wistar (Han) Rats.
870.4300	48604446	SYN545192: 104 week rat dietary carcinogenicity study with combined 52 week toxicity study
870.4200	48604448	SYN545192 - 80 week mouse dietary carcinogenicity study
870.3800	48604449	SYN545192 – Two-Generation Reproduction Toxicity Study in the Han Wistar Rat
870.3700	48604451	SYN545192 - Prenatal Developmental Toxicity Study in the Han Wistar Rat

870.3700	48604453	SYN545192 – A Prenatal Developmental Toxicity Study in New Zealand White Rabbits
870.6200	48604455	SYN545192 - Acute Oral (Gavage) Neurotoxicity Study in the Rat
870.6200	48604457	SYN545192 - 13 Week Dietary Neurotoxicity Study in Rats.
870.7600	48604460	SYN545192 EC Formulation (A17056F) – In Vivo Dermal Absorption in the Rat.
870.7800	48604461	SYN545192 – A 28-Day Dietary Immunotoxicity Study in CD-1 Female Mice
Non-guideline	48604553	SYN545192: 14 day dietary thyroid mode of action study in rats with a 63 day recovery period
Non-guideline	48604554	SYN545192: Effect on Hepatic UDPglucuronosyltransferase activity towards thyroxine as substrate after dietary administration for up to 28 days to male rat
Non-guideline	48604555	SYN545192 - Effect on rat thyroid peroxidase activity in vitro
Non-guideline	48604556	SYN545192: A histological extension study of male thyroid tissue from rat toxicity study
870.7485	48604579	SYN545192 – Pharmacokinetics of Total radioactivity in the Rat following Intravenous and Oral Administration of [^{14}C]-SYN545192
Non-guideline	48604588	SYN545192: Investigative 28 day dietary study in rats with interim kills
870.3050	48836614	CSAA798670 - Repeated Dose 28-Day Oral Toxicity Study in rodents
870.7600	48604563 48604564	SYN545192 EC (A17056F) – *In Vitro* Absorption through Rat Epidermis Using [^{14}C]-Radiolabelled SYN545192. SYN545192 EC (A17056F) – *In Vitro* Absorption through Human Epidermis Using [^{14}C]-Radiolabelled SYN5451

B.6.4.3. Study type: NOA449109 – *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay; OECD 471 (1997), OPPTS 870.5100 (1998), EC 440/2008 B.13/14 (2008)

Test Material (purity): NOA449109 (Metabolite of SYN545192) (99.5 %, estimated error ± 0.3 %)

Synonyms: CA4925

Sponsor: Syngenta Crop Protection, LLC, Greensboro, USA.

Report Number: 1412200

Executive Summary: In a reverse gene mutation assay in bacteria, strains TA1535, TA1537, TA98, and TA100 TA1537 of *S. typhimurium* and strain *WP2 uvrA* pKM101 and WP2 pKM101 of *E. coli* were exposed to NOA449109 metabolite (99.5%) in DMSO at concentrations 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate *w*ithout S9 mix) or 33; 100; 333; 1000; 2500; and 5000 µg/plate with S9 mix.

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No precipitation of the test item was observed up to the highest concentration tested.
Toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred at concentrations of 2500 and 5000 µg/plate in strains WP2 *uvrA* pKM101 and only at concentration of 5000 µg/plate in strains WP2 pKM101 both experiments were conducted in absence of metabolic activation.

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with NOA449109 at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.
Therefore, NOA449109 is considered to be non-mutagenic in this Salmonella typhimurium and Escherichia coli reverse mutation assay.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Materials and Methods

Materials

Test material:
Description:
Lot/Batch #: AMS 1397/1
Purity: 99.5 % (estimated error ± 0.3 %).
CAS #:
Stability in Solvent: Not indicated by the sponsor

Control materials:
Negative: Concurrent untreated and solvent controls were performed.

Positive: Non-activation:
Sodium azide 10 µg/plate TA1535, TA100
4-nitro-o-phenylene-diamine 10 µg/plate TA 98; 50 µg/plate TA 1537
Methyl methane sulfonate 3 µL/plate WP2 *uvrA* pKM101, WP2 pKM101

Activation:
2-Aminoanthracene 2.5 µg/plate (TA1535, TA1537, TA98, TA100);
10 µg/plate (WP2 *uvrA* pKM101, WP2 pKM101)

Activation: S9 derived from

X	Induced	X	β-naphthoflavone	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
					Hamster		Other
					Other		

Describe S9 mix composition:
8mM MgCl2
33 mM KCl
5 mM Glucose-6-phosphate
4 mM NADP
in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.
The amount of S9 supernatant was 10% v/v in the S9 mix

Test organisms:
S. typhimurium strains: TA1535, TA1537, TA98, and TA100
Escherichia coli strains: WP2 *uvrA* pKM101 and WP2 pKM101

Test compound concentrations used:
Preliminary Test:
In the pre-experiment the concentration range of the test item was 3 - 5000 µg/plate. Since the criteria mentioned above were met, the pre-experiment is reported as Experiment I. Since minor toxic effects were observed seven (without S9 mix) and six (with S9 mix) concentrations were tested in experiment II and 5000 µg/plate were chosen as maximal concentration.

Main Assay (all strains):

Pre-Experiment/Experiment I:

With or Without S9 mix: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II:

Without S9 mix: 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
With S9 mix: 33; 100; 333; 1000; 2500; and 5000 µg/plate

Test performance

Protocol. In each experiment, for each strain and dose level (including controls) three plates were used.
The following materials were mixed in a test tube and poured onto the selective agar plates:

100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 µL S9 mix (for test with metabolic activation) or S9 mix substitution buffer* (for test without metabolic activation),
100 µL Bacteria suspension (cf. test system, pre-culture of the strains),
2000 µL Overlay agar

For the pre-incubation method 100 JL test solution (solvent or reference mutagen solution (positive control), 500 µL S9 mix / S9 mix substitution buffer* and 100 µL bacteria suspension were mixed in a test tube and incubated at 37 °C for 60 minutes. After preincubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on selective agar plates.
After solidification the plates were incubated upside down for 48 - 72 hours at 37°C in the dark.

* Substitution buffer: 7 parts of the 100 mM sodium-ortho-phosphate-buffer pH 7.4 with 3 parts of KCl solution 0.15 M.

Statistics – Not conducted.

Results

Preliminary cytotoxicity assay – No cytotoxicity was observed in any strain at any dose level, but precipitation was evident at 5000 µg/plate without S9 mix. A two-fold increase or more above solvent control in the mean number of revertant colonies was not observed in any strain in either the absence or presence of metabolic activation. Therefore, a range of doses up to a maximum of 5000 µg/plate (limit dose) should be used in the main assay.

Mutagenicity assay – Test material concentrations of 20.6 to 5000 µg/plate (Experiment I) and 156 to 5000 µg/plate (Experiment II) failed to elicit a two-fold or greater increase above solvent control in the mean number of revertant colonies in any strain with or without metabolic activation. No cytotoxicity was observed in any strain at any dose level, but precipitation was evident at 5000 µg/plate without S9 mix in both experiments.

Table 1. Number of Revertant Colonies (group mean & standard deviation), without activation test
Experiment I

Without Activation						
	Revertant Colony Counts (Mean ±SD)					
Dose (µg/plate)	TA1535	TA1537	TA 98	TA100	WP2 pKM101	WP2 uvrA pKM101
DMSO	11 ± 2	16 ± 5	25 ± 8	131 ± 5	215 ± 6	374 ± 15
CONTROL	15 ± 1	10 ± 3	28 ± 9	148 ± 8	237 ± 1	372 ± 13
3	11 ± 3	15 ± 3	24 ± 4	131 ± 19	242 ± 38	370 ± 4
10	13 ± 7	15 ± 4	26 ± 6	138 ± 2	225 ± 24	361 ± 9
33	12 ± 3	18 ± 6	26 ± 4	120 ± 8	217 ± 6	341 ± 7
100	11 ± 4	16 ± 4	23 ± 7	122 ± 22	213 ± 12	336 ± 50
333	16 ± 3	17 ± 4	20 ± 1	146 ± 20	214 ± 34	403 ± 18
1000	13 ± 3	17 ± 4	23 ± 3	141 ± 10	156 ± 39	374 ± 17
2500	12 ± 4	16 ± 5	23 ± 4	124 ± 18	109 ± 7	256 ± 8
5000	12 ± 4	14 ± 1	23 ± 4	130 ± 23	57 ± 7	149 ± 8
Positive						
NaN3 10 µg	1643 ± 108			1886 ± 71		
4-NOPD 10 µg			405 ± 31			
4-NOPD 50 µg		79 ± 7				
MMS 3.0 µL					2897± 198	3012 ± 69
With Activation						
Dose (µg/plate)	TA1535	TA1537	TA 98	TA100	WP2 pKM101	WP2 uvrA pKM101
DMSO	18 ± 2	28 ± 5	40 ± 7	127 ± 14	230 ± 9	423 ± 34
CONTROL	20 ± 3	28 ± 2	43 ± 8	155 ± 10	280 ± 11	433 ± 29
3	17 ± 2	29 ± 2	39 ± 6	132 ± 18	279 ± 32	380 ± 16
10	22 ± 7	26 ± 4	38 ± 11	130 ± 5	237 ± 5	384 ± 33

33	18 ± 4	24 ± 4	37 ± 4	120 ± 3	240 ± 28	355 ± 28
100	22 ± 2	23 ± 6	39 ± 9	134 ± 27	226 ± 27	384 ± 44
333	19 ± 9	25 ± 4	46 ± 4	142 ± 11	231 ± 39	391 ± 28
1000	19 ± 6	28 ± 5	34 ± 9	138 ± 6	193 ± 15	346 ± 5
2500	17 ± 3	28 ± 2	30 ± 6	147 ± 13	169 ± 29	368 ± 51
5000	18 ± 4	24 ± 4	30 ± 9	144 ± 29	158 ± 22	288 ± 48
Positive						
2-AA 2.5 µg	282 ± 23	227 ± 14	1727 ± 317	1601 ± 217		
2-AA 10 µg					1960 ± 66	1841 ± 40

Table 2. Number of Revertant Colonies (group mean / standard deviation), test Experiment II

Without Activation						
	Revertant Colony Counts (Mean ±SD)					
Dose (µg/plate)	TA1535	TA1537	TA 98	TA100	WP2 pKM101	WP2 uvrA pKM101
DMSO	17 ± 6	11 ± 4	34 ± 6	138 ± 9	215 ± 6	424 ± 20
CONTROL	21 ± 5	14± 6	49± 9	163 ± 6	247 ± 1	441 ± 54
10	14 ± 5	10 ± 4	33 ± 6	132 ± 25	241 ± 30	421 ± 30
33	17 ± 5	11 ± 2	35 ± 6	127 ± 5	206 ± 9	414 ± 41
100	17 ± 3	11 ± 5	35 ± 5	135 ± 5	209 ± 13	381 ± 20
333	15 ± 5	9 ± 1	31 ± 10	131 ± 3	213 ± 13	394 ± 10
1000	17 ± 3	11 ± 2	33 ± 6	128 ± 20	144 ± 7	370 ± 10
2500	13 ± 5	9 ± 3	23 ± 4	136 ± 11	86 ± 14	261 ± 30
5000	16 ± 6	7 ± 2	19 ± 7	109 ± 23	54 ± 9	173 ± 4
Positive						
NaN3 10 µg	1892 ± 98			1772 ± 190		
4-NOPD 10 µg			341 ± 33			

4-NOPD 50 µg		78 ± 3				
MMS 3.0 µL					3428 ± 361	2718 ± 134
With Activation						
	Revertant Colony Counts (Mean ±SD)					
Dose (µg/plate)	TA1535	TA1537	TA 98	TA100	WP2 pKM101	WP2 *uvr*A pKM101
DMSO	23 ± 2	15 ± 1	42 ± 8	141 ± 14	244 ± 8	449 ± 38
CONTROL	29 ± 5	23 ± 6	58 ± 6	168 ± 18	287 ± 4	456 ± 14
33	24 ± 5	15 ± 5	47 ± 8	161 ± 15	251 ± 2	492 ± 92
100	23 ± 1	15 ± 2	43 ± 2	152 ± 10	219 ± 10	427 ± 8
333	22 ± 2	16 ± 1	44 ± 6	151 ± 4	249 ± 15	440 ± 37
1000	24 ± 8	18 ± 3	40 ± 4	161 ± 13	237 ± 25	429 ± 19
2500	26 ± 5	19 ± 2	45 ± 4	151 ± 8	191 ± 24	440 ± 21
5000	21 ± 2	19 ± 2	50 ± 5	130 ± 11	142 ± 11	416 ± 41
Positive						
2-AA 2.5 µg	336 ± 4	318 ± 51	1373 ± 27	2701 ± 128		
2-AA 10 µg					1915 ± 59	1915 ± 59

Discussion

Investigators' conclusions – "In experiment I and II of the reverse mutation test with NOA449109, a two-fold or greater increase in the mean number of revertant colonies compared to the DMSO control was not observed in any strain at any dose either in the presence or absence of a metabolic activation system. It is concluded that NOA449109 is not mutagenic to the bacteria under the conditions used in this test."

Reviewer comments - The reviewer agrees with the study author's conclusion. There was no evidence of induced mutant colonies over background. NOA449109 did not induce mutagenicity in any of the six bacterial tester strains in either the presence or absence of metabolic activation according to the described methods.
This reviewer noted that cytotoxicity (observed as a reduction in the number of revertantes) in 5000 µg/plate in strains WP2 pKM101 and WP2 uvrA in the absence of metabolic activation of the experiments I and II (below the factor 0.5), however, the research concluded that no cytotoxicity was observed in any strain at any dose level.

Anyway, considering the high dose of the compound. This observation has no impact on the results.

Study deficiencies – Designated strain-specific positive control compounds were not used; however, the positive control substances elicited the expected response and historical positive control data were submitted and supported the use of the specified positive control agents.

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Study type: CSCD465008 – *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay; OECD 471 (1997), OPPTS 870.5100 (1998), EC 2000/32 (2000).

Test Material (purity): CSCD465008 (94 %)

Synonyms: CSCD465008 (Metabolite of SYN520453)

Sponsor: Syngenta Crop Protection, Inc. Greensboro, USA.

Report Number: 1129601

Executive Summary: In a reverse gene mutation assay in bacteria, strains TA1535, TA98, TA1537 and TA100 of *Salmonella typhimurium* and strains *WP2 uvrA* pKM101 and WP2 pKM101 of *Escherichia coli* were exposed to CSCD465008 (Metabolite of SYN520453) (94%) in DMSO at concentrations 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate or 33; 100; 333; 1000; 2500; and 5000 µg/plate with S9 mix.

The plates incubated with the test item showed normal background growth with and without metabolic activation in both independent experiments. No precipitation of the test item was observed, neither in the overlay agar in the test tubes nor on the incubated agar plates. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with CSCD465008 at at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

In experiment II, the laboratory´s historical control range was exceeded in the untreated control of strain WP2 pKM 101 with and without metabolic activation. These elevated colony counts were considered to be the result of biologically irrelevant fluctuations and had no detrimental impact on the outcome of the study.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, CSCD465008 is considered to be non-mutagenic in this *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Materials and Methods

Study design and methods

Study experimentation dates - Start: January 02, 2008 End: June 12, 2008

Materials

Test material:

Description: Beige solid
Lot/Batch #: MES-103/1
Purity: 94 %
CAS #:
Stability in Solvent: Not indicated by the sponsor

Control materials:

Negative: Concurrent untreated and solvent controls were performed.

Positive: Non-activation:

- Sodium azide (NaN_3) 10 µg/plate TA1535, TA100

4-nitro-o-phenylene-diamine (4-NOPD) 10 µg/plate in TA 98; 50 µg/plate in TA 1537

- Methyl methane sulfonate (MMS) 3 µL/plate. WP2 *uvrA* (pKM101), WP2 (pKM101)

Activation:

- 2-Aminoanthracene (2-AA) 2.5 µg/plate (TA1535, TA1537, TA98, TA100); 10 µg/plate (WP2 *uvrA* pKM101, WP2 pKM101)

Activation:

S9 mix composition:

8mM MgCl2
33 mM KCl
5 mM Glucose-6-phosphate
5 mM NADP

In 100 mM sodium-ortho-phosphate-buffer, pH 7.4.
The amount of S9 supernatant was 10% v/v in the S9 mix

Test organisms:
Salmonella typhimurium strains: TA1535, TA1537, TA98, and TA100
Escherichia coli strains: WP2 *uvrA* pKM101 and WP2 pKM101

Test compound concentrations used:

Preliminary Test:

To evaluate the citoxicity of the test item a pre-experiment was performed with strains TA 1535, TA 1537, TA 98, TA 100, WP2 uvrA pKM 101, and WP2 pKM 101. Eight concentrations were tested for toxicity and mutation induction with three plates each.

Main Assay (all strains):

Pre-Experiment/Experiment I:
3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II:
33; 100; 333; 1000; 2500; and 5000 µg/plate

Test performance

Protocol. In each experiment, for each strain and dose level (including controls) three plates were used.
The following materials were mixed in a test tube and poured onto the selective agar plates:
100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 µL S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µL Bacteria suspension (test system, pre-culture of the strains)
2000 µL Overlay agar

For the pre-incubation method 100 µL test solution, 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and incubated at 37 °C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on selective agar plates.
After solidification the plates were incubated upside down for 48 - 72 hours at 37°C in the dark.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

Statistics – According to the OECD guideline 471, a statistical analysis of the data is not mandatory.

Results

Preliminary assay – No citoxicity was observed in any strain at any dose level. Since no toxic effects were observed, 5000 µg/plate was chosen as maximal concentration.

Mutagenicity assay – In experiment II, the laboratory´s historical control range was exceeded in the untreated control of strain WP2 pKM 101 with and without metabolic activation. These elevated colony counts were considered to be the result of biologically irrelevant fluctuations and had no detrimental impact on the outcome of the study. No toxicity was observed in any strain at any dose level.

Table 1. Number of Revertant Colonies (group mean & standard deviation), without activation test
Experiment I

Without Activation						
	Revertant Colony Counts (Mean ±SD)					
Dose (µg/plate)	TA1535	TA1537	TA 98	TA100	WP2 pKM101	WP2 uvrA pKM101
DMSO	13 ± 3	15 ± 1	19 ± 4	116 ± 5	323 ± 9	226 ± 16
CONTROL	11 ± 2	12 ± 4	19 ± 6	102 ± 6	305 ± 4	228 ± 24
3	11 ± 5	11 ± 3	18 ± 3	102 ± 6	216 ± 3	207 ± 13
10	12 ± 2	14 ± 2	19 ± 3	94 ± 6	297 ± 4	226 ± 14
33	14 ± 2	17 ± 2	26 ± 5	95 ± 5	276 ± 30	219 ± 17
100	15 ± 1	12 ± 3	24 ± 2	99 ± 12	323 ± 13	211 ± 21
333	14 ± 2	15 ± 3	21 ± 3	99 ± 10	294 ± 22	208 ± 3
1000	14 ± 1	14 ± 2	20 ± 3	111 ± 18	315 ± 10	222 ± 10
2500	14 ± 1	12 ± 3	21 ± 2	101 ± 6	322 ± 6	223 ± 7
5000	14 ± 2	14 ± 4	24 ± 3	115 ± 11	343 ± 22	224 ± 5
Positive						
NaN3 10 µg	1835 ± 40			2095 ± 43		
4-NOPD 10 µg			332 ± 15			
4-NOPD 50 µg		92 ± 12				
MMS 3.0 µL					3882± 339	4001 ± 340
With Activation						
Dose (µg/plate)	TA1535	TA1537	TA 98	TA100	WP2 pKM101	WP2 uvrA pKM101
DMSO	14 ± 2	16 ± 1	32 ± 2	122 ± 16	360 ± 23	230 ± 9
CONTROL	14 ± 3	16 ± 5	39 ± 3	102 ± 19	347 ± 43	276 ± 35
3	14 ± 3	20 ± 3	32 ± 3	106 ± 4	369 ± 24	260 ± 20
10	12 ± 2	20 ± 2	33 ± 10	114 ± 7	341 ± 13	250 ± 25
33	16 ± 1	16 ± 3	31 ± 3	121 ± 9	316 ± 48	265 ± 18

100	17 ± 6	19 ± 4	29 ± 4	126 ± 12	324 ± 73	255 ± 10
333	15 ± 3	18 ± 2	31 ± 4	103 ± 3	379 ± 68	241 ± 19
1000	14 ± 1	21 ± 2	32 ± 2	105 ± 9	345 ± 23	234 ± 18
2500	13 ± 2	20 ± 3	31 ± 4	110 ± 15	323 ± 3	190 ± 33
5000	17 ± 2	22 ± 2	31 ± 6	115 ± 10	333 ± 23	252 ± 16
Positive						
2-AA 2.5 µg	203 ± 6	119 ± 14	948 ± 82	1420 ± 141		
2-AA 10 µg					2050 ± 78	3848 ± 67

Table 2. Number of Revertant Colonies (group mean / standard deviation), test Experiment II

Without Activation						
	Revertant Colony Counts (Mean ±SD)					
Dose (µg/plate)	TA1535	TA1537	TA 98	TA100	WP2 pKM101	WP2 uvrA pKM101
DMSO	16 ± 4	14 ± 2	28 ± 6	184 ± 22	325 ± 20	246 ± 31
CONTROL	18 ± 3	18 ± 3	26 ± 8	190 ± 13	369 ± 5	270 ± 26
33	18 ± 1	13 ± 2	26 ± 3	180 ± 11	325 ± 27	245 ± 28
100	15 ± 3	13 ± 4	28 ± 2	191 ± 16	318 ± 10	261 ± 23
333	18 ± 3	12 ± 2	28 ± 3	191 ± 6	321 ± 9	226 ± 10
1000	17 ± 4	14 ± 2	27 ± 7	200 ± 25	338 ± 4	251 ± 24
2500	13 ± 2	16 ± 1	27 ± 3	213 ± 18	335 ± 19	235 ± 25
5000	16 ± 3	15 ± 2	29 ± 2	199 ± 3	367 ± 9	241 ± 20
Positive						
NaN3 10 µg	2024 ± 78			1795 ± 69		
4-NOPD 10 µg			361 ± 22			
4-NOPD 50 µg		135 ± 5				
MMS 3.0 µL					1369 ± 203	2511 ± 239

With Activation						
	Revertant Colony Counts (Mean ±SD)					
Dose (µg/plate)	TA1535	TA1537	TA 98	TA100	WP2 pKM101	WP2 *uvr*A pKM101
DMSO	14 ± 4	13 ± 2	32 ± 5	190 ± 39	379 ± 18	273 ± 15
CONTROL	16 ± 3	17 ± 3	36 ± 1	201 ± 13	401 ± 39	319 ± 5
33	15 ± 2	15 ± 4	37 ± 7	161 ± 34	380 ± 11	268 ± 9
100	14 ± 1	17 ± 2	32 ± 3	154 ± 14	353 ± 29	253 ± 34
333	14 ± 42	16 ± 3	35 ± 8	150 ± 7	289 ± 15	272 ± 13
1000	15 ± 2	13 ± 3	34 ± 1	148 ± 8	403 ± 22	276 ± 24
2500	14 ± 4	15 ± 6	34 ± 6	143 ± 1	404 ± 22	275 ± 19
5000	15 ± 2	14 ± 4	33 ± 5	149 ± 10	401 ± 31	220 ± 22
Positive						
2-AA 2.5 µg	260 ± 4	176 ± 29	1039 ± 79	1240 ± 140		
2-AA 10 µg					2524 ± 69	1989 ± 82

Discussion

Investigators' conclusions – "In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used."

Reviewer comments - The reviewer agrees with the study author's conclusion. There was no evidence of induced mutant colonies over background. CSCD465008 did not induce mutagenicity in any of the bacterial tester strains in either the presence or absence of metabolic activation according to the described methods. This reviewer noted that in experiment II, the laboratory´s historical control range was exceeded in the untreated control of strain WP2 pKM 101 with and without metabolic activation, however, the research concluded that these elevated colony counts were the result of biologically irrelevant fluctuations and had no detrimental impact on the outcome of the study.

Study deficiencies – This reviewer found no deficiencies

Study type: *In vitro* mammalian clastogenicity – Chromosome Aberration assay. OPPTS 870.5375; OECD 473.

Test Material (purity): CSAA798670 (97.4 %)

Citation: CSAA798670 – Chromosome Aberration Test in in *Vitro.* Harlan Cytotest Cell Research GmbH, Germany. Laboratory report number: 1266902. Study report date: November 26, 2009.

Sponsor: Syngenta Crop Protection, LLC, Greensboro, USA

Executive Summary:

In a mammalian cell cytogenetics/chromosome aberration assay, human lymphocyte cultures were exposed to CSAA798670 (97.4%) in DMSO at concentrations of 591.0, 1034.3, 1810.0 µg/mL with and without metabolic activation for a 4 and 22 hour exposure period. Cells were prepared following an 18 hour recovery period (22 hour preparation interval).

In each experimental group two parallel cultures were analyzed. Per culture 100 metaphase plates were scored for structural chromosomal aberrations.

In the absence and presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration. In both independent experiments, no biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment with the test item.
No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.

In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce structural chromosomal aberrations in human lymphocytes *in vitro*.
Therefore, CSAA798670 is considered to be non-clastogenic in this chromosome aberration test in the absence and presence of metabolic activation when tested up to the highest required concentration.

This study is classified as acceptable. This study satisfies the requirement for Test Guideline [In vitro mammalian cytogenetics chromosome aberration assay] OPPTS 870.5375; OECD 473 for in vitro cytogenetic mutagenicity data.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. Materials and Methods

A. Materials

1. **Test material:** CSAA798670
   Description: Not provided
   Lot/Batch #: LT-DFPA09001

Purity: 97.4 %

Solvent used: DMSO

2. Control materials:

Negative: DMSO

Solvent (final conc): 0.5% DMSO in culture medium

Positive: **With activation:** CPA; cyclophosphamide. Final concentration: 7.5 µg/mL
Without activation: EMS, ethylmethane sulfonate Final concentration: 825 µg/mL (experiment I), 660 µg/mL (experiment II)

3. Activation: S9 derived from

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
		X	naphthoflavone		Hamster		Other
			5,6-benzoflavone		Other		

Described S9 mix composition:

8 mM $MgCl_2$
33 mM KCl
5 mM glucose-6-phosphate
4 mM NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

4. Test cells: mammalian cells in culture: Human lymphocytes
Media: DMEM: F12 (Dulbecco's modified eagle medium/ Ham's F12 medium; mixture 1:1), containing 10 % FCS (fetal calf serum). The antibiotic solution contains 10,000 U/mL penicillin and 10,000 µg /mL streptomycin. Additionally, the medium was supplemented with Phytohemagglutinin (final concentration 3 µg /mL), the anticoagulant heparin (25,000 U.S.P.-U/mL), and HEPES (final concentration 10 Mm).

5. Test compound concentrations used (µg/mL):

Schedule

	Without S9 mix		With S9 mix	
	Experiment I	Experiment II	Experiment I	Experiment II
Exposure period	4 hrs	22 hrs	4 hrs	4 hrs
Recovery	18 hrs	-	18 hrs	-
Preparation interval	22 hrs	22 hrs	22 hrs	22 hrs

Experiment I:	Non-activated (4 hr exposure): 591.0, 1034.3, 1810.0 µg/mL
	Activated (4 hr exposure): 591.0, 1034.3, 1810.0 µg/mL
Experiment II:	Non-activated (22 hr exposure): 591.0, 1034.3, 1810.0 µg/mL
	Non-activated (4 hr exposure): 591.0, 1034.3, 1810.0 µg/mL

B. Test performance

1. Preliminary cytotoxicity assay – A preliminary cytotoxicity test was performed to determine the concentrations to be used in the mutagenicity assay. Cytotoxicity is characterized by the percentages of mitotic suppression in comparison to the controls by counting 1000 cells per culture in duplicate.
The experimental conditions in this pre-test phase were identical to those required for the mutagenicity assay.
The pre-test phase was performed with 10 concentrations of the test item and a solvent and positive control. All cell cultures were set up in duplicate. Exposure time was 4 hours (with and without S9 mix). The preparation interval was 22 hours after start of the exposure.

2. Cytogenetic assay -

a. Cell exposure time	Test Material	Solvent Control	Positive Control
Non-activated:	4 or 22 h	4 or 22 h	4 or 22 h
Activated:	4 or 22 h	4 or 22 h	4 or 22 h

b. Spindle inhibition

Inhibition used/concentration:	0.2 µg/mL colcemid
Administration time:	2 hours (before cell harvest)

c. Cell harvest time after termination of treatment	Test Material	Solvent Control	Positive Control
Non-activated:	22 h	22 h	22 h
Activated:	22 h	22 h	22 h

d. Details of slide preparation – Three hours before harvesting, colcemid was added to the cultures (final concentration 0.2 µg /mL). The cultures were harvested by centrifugation 22 hrs after beginning of treatment. The supernatant was discarded and the cells were resuspended in approximately 5 mL hypotonic solution (0.0375 M KCl). The cell suspension was then allowed to stand at 37 °C for 20 to 25 minutes. After removal of the hypotonic solution by centrifugation the cells were fixed with a mixture of methanol and glacial acetic acid (3 parts plus 1 part). At least two slides per experimental group were prepared by dropping the cell suspension onto a clean microscope slide. The cells for evaluation of cytogenetic damage were stained with Giemsa.

e. Metaphase analysis

No. of cells examined : 1000 per culture for determination of mitotic index

Scored for structural? [X] Yes [ ] No

Scored for numerical? [X] Yes (polyploid) [ ] No

f. Evaluation criteria – A test item was classified as non-mutagenic if:

- The number of induced structural chromosome aberrations in all evaluated dose groups was in the range of laboratory historical control data.
- No significant increase of the number of structural chromosome aberrations was observed.

A test item was classified as mutagenic if:

- The number of induced structural chromosome aberrations was not in the range of laboratory historical control data.
- either a concentration-related or a significant increase of the number of structural chromosome aberrations was observed.

2. <u>Statistics</u> – "Statistical significance at the five per cent level ($p < 0.05$) for aberration frequency was evaluated by means of the Fisher's exact test. Evaluation was performed only for cells carrying aberrations excluding gaps."

II. Reported Results

<u>Cytogenetic assay</u> – In the absence and presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration.
In both independent experiments, no biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment with the test item. No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures. Appropriate mutagens were used as positive controls. They induced statistically significant increases ($p < 0.05$) in cells with structural chromosome aberrations.

In both experiments, in the absence and presence of S9 mix, no precipitation, relevant increase or decrease in the osmolarity or pH value of the test item in the culture medium was observed. In the absence and presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration.
In both experiments, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. The aberration rates of the cells after treatment with the test item (0.5 – 2.5 % aberrant cells, excluding gaps) were close to the solvent control values (1.0 – 3.0 % aberrant cells, excluding gaps) and were within the range of the laboratory's historical solvent control data.
No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.

Experiment I. Cultures exposed for 4 hours without S9 mix. Preparation interval: 22 hrs.

Test concentration in µg/mL	Mitotic indices in % of control	Including gaps	Aberrant cells in % excluding gaps	Carrying exchanges
Solvent control	100.0	1.0	1.0	0.0
Positive control	71.3	16.0	15.0 [S]	4.5
591.0	105.8	1.0	1.0	0.0

1034.3	91.6	2.5	2.5	0.0
1810.0	82.2	2.0	2.0	0.0

Experiment II. Cultures exposed for 22 hours without S9 mix. Preparation interval: 22 hrs.

Test concentration in µg/mL	Mitotic indices in % of control	Including gaps	Aberrant cells in % excluding gaps	Carrying exchanges
Solvent control	100.0	3.0	3.0	0.0
Positive control	50.2	22.0	22.0 [S]	5.5
591.0	112.0	1.5	1.5	0.0
1034.3	92.0	1.5	1.0	0.0
1810.0	80.9	2.0	1.5	0.0

Experiment I Cultures exposed for 4 hours with S9 mix. Preparation interval: 22 hrs.

Test concentration in µg/mL	Mitotic indices in % of control	Including gaps	Aberrant cells in % excluding gaps	Carrying exchanges
Solvent control	100.0	2.0	2.0	0.0
Positive control	47.2	11.5	11.0 [S]	1.5
591.0	85.3	1.5	1.0	0.0
1034.3	93.1	2.0	1.5	0.0
1810.0	93.4	2.5	2.0	0.0

Experiment II Cultures exposed for 4 hours with S9 mix. Preparation interval: 22 hrs.

Test concentration in µg/mL	Mitotic indices in % of control	Including gaps	Aberrant cells in % excluding gaps	Carrying exchanges
Solvent control	100.0	1.5	1.5	0.0
Positive control	58.1	20.5	20.0 [S]	1.5
591.0	104.2	1.0	0.5	0.0
1034.3	108.5	1.0	1.0	0.0
1810.0	101.9	0.5	0.5	0.0

S: Aberration frequency statistically significant higher than corresponding values

III. Discussion

A. <u>Investigators' conclusions</u> – "In conclusion, it can be stated that under the experimental conditions reported, the test item CSAA798670 did not induce structural chromosomal aberrations in human lymphocytes in *vitro* in the absence and presence of metabolic activation, when tested up to the highest required concentration"

B. <u>Reviewer comments</u> - The reviewer agrees with the study author's conclusion. Under the experimental conditions reported, the test item CSAA798670 did not induce structural chromosomal aberrations in human lymphocytes in *vitro* in the absence and presence of metabolic activation when tested up to cytotoxic or precipitating concentrations. The positive control materials induced the expected response.

C. <u>Study deficiencies</u> – none

Study Type: CSAA798670 – Cell Mutation Assay at the Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y Cells**.** Wollny H-E., Germany. Laboratory report number: 1266901. Study report date: 30-November-2009. OECD 476 (1997); EPA OPPTS 870.5300 (1998); 2008/440/EC B.17 (2008).

Test Material (purity): 97.4%

Sponsor: Syngenta Crop Protection, LLC. Greensboro, USA.

Executive Summary: In a mammalian cell gene mutation assay at the thymidine kinase (TK) locus, L5178Y mouse lymphoma cells cultured *in vitro* were exposed to CSAA798670 (97.4%) in DMSO. The assay was performed in two independent experiments, using two parallel cultures each. Both main experiments were performed with and without liver microsomal activation and a treatment period of 4 hours. The main experiments were evaluated with the following concentrations: Experiments I and II (with and without S9 mix): 113.1, 226.3, 425.5, 905, 1810 µg/mL.

CSAA798670 was tested up to cytotoxic and/or precipitating concentrations. No relevant toxic effects indicated by a relative cloning efficiency 1 (survival) or a relative total growth (RTG) of less than 50% in both cultures occurred in experiment I and II up to the maximum concentration with and without metabolic activation. No substantial and reproducible dose dependent increase of the mutation frequency was observed in experiment I and II up to the maximum concentration with and without metabolic activation. The threshold of 126 above the corresponding solvent control was not reached or exceeded at any test point with and without metabolic activation.

In conclusion it can be stated that during the mutagenicity test described and under the experimental conditions reported, the test item CSAA798670 did not induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y in the absence and presence of metabolic activation.

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. Materials and Methods

A. Materials

1. Test material: CSAA798670

Description: Light-brown powder

Lot/Batch #: LT-DFPA09001

Purity: 97.4%

CAS #:

Solvent used: DMSO

2. Control materials:

Negative: DMSO

Positive: Non-activation: Methyl methane sulfonate (MMS)

Activation: Cyclophosphamide (CPA)

3. Activation: S9 derived from

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	ß-naphthoflavone		Other		

S9 mix composition

The protein concentration of the S9 preparation was 35.6 mg/mL in the pre-experiment and in experiments I. In experiment II the protein concentration was 32.3 mg/mL (Lot. No.: 020709)

18 mM $MgCl_2$
33 mM KCl
5 mM glucose-6-phosphate
4 mM NADP

In 100 mM sodium-ortho-phosphate-buffer, pH 7.4.
The concentration in the final test medium was 5 % (v/v).
To 0.5 mL S9 mix components, 0.5 mL of S9 fraction was added (50% v/v) to complete the S9 mix.

4. Test cells: mammalian cells in culture

X	mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells		list any others

Medium: RPMI 1640-HAP

Prior to mutagenicity testing the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with: hypoxanthine (1.0×10^{-4} M), aminopterin (2.0×10^{-7} M) and thymidine (1.6×10^{-5} M). The incubation of the cells in HAT-medium was followed by a recovery period of 2 days in RPMI 1640 medium containing: hypoxanthine (1.0×10^{-4} M) and thymidine

(1.6×10^{-5} M).

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

5. **Locus Examined:** X Thymidine kinase (TK) Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) Na^+/K^+ ATPase

Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		fluorodeoxyuridine (FdU)		6-thioguanine (6-TG)		
	X	trifluorothymidine (TFT)				

6. **Test compound concentrations used**:

The main experiments were evaluated at the following concentrations:

Experiment I:

without S9 mix: 113.1, 226.3, 425.5, 905, 1810 µg/mL
with S9 mix: 113.1, 226.3, 425.5, 905, 1810 µg/mL

Experiment II:

- without S9 mix: 113.1, 226.3, 425.5, 905, 1810 µg/mL
- with S9 mix: 113.1, 226.3, 425.5, 905, 1810 µg/mL

B. Test performance

1. Cell treatment -

a. 1 x 10^7 cells were exposed to test compound, negative/solvent or positive control substance for 4 hours (non-activated and activated).

b. After washing, cells were cultured for 2 days (expression and growth period) before cell selection.

c. After expression, the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4×10^3 cells in selective medium

with TFT. The viability (cloning efficiency 2) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37°± 1.5 °C in 4.5 % CO2/95.5 % water saturated air for 10 - 15 days. Then the plates were evaluated.

Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the large colonies). The survival rate and viability were determined based on the Poisson distribution method. The zero term of the Poisson distribution, [P(0)] method, was used. The mutation frequency was derived from the cloning efficiency under selective conditions compared to the corresponding viability under non-selective conditions.

2. Statistics – A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT_11 statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend was judged as significant whenever the p-value (probability value) was below 0.05. Both, biological relevance and statistical significance were considered together.

3. Evaluation criteria

- A test item was classified as mutagenic if the induced mutation frequency reproducibly exceeded a threshold of 126 colonies per 106 cells above the corresponding solvent control or negative control, respectively.
- A relevant increase of the mutation frequency should be dose-dependent.
- A mutagenic response was considered to be reproducible if it occurred in both parallel cultures.
- In the evaluation of the test results the historical variability of the mutation rates in negative and vehicle controls and the mutation rates of all negative and vehicle controls were taken into consideration.
- Results of test groups were generally rejected if the relative total growth and the cloning efficiency 1 is less than 10 % of the vehicle control unless the exception criteria specified by the IWGT recommendations were fulfilled.
- Whenever a test item was considered mutagenic, the ratio of small versus large colonies was used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency was accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.

II. Reported Results

A. Pre-experiment– A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. Both pH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation. 1×10^7 cells

were exposed to each concentration of the test item for 4 hours with and without metabolic activation. Following treatment the cells were washed twice by centrifugation (425 g, 10 min) and resuspended in "saline G". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to 3×10^5 cells/mL, if necessary.

Table 1. Cell count, pre-experiment

	Conc. µg per mL	S9 mix	Number of cells per mL (4h after treatment)	Number of cells per mL (24h after treatment)	Number of cells per mL (48h after treatment)	Total suspension growth TSG	Relative suspension growth RSG
Solvent control w/ DMSO		-	285200	939000	1191000	13.1	100.0
Test item	14.1	-	303800	1048000	1128000	13.0	99.2
Test item	28.3	-	273000	989000	1081800	13.1	99.9
Test item	56.6	-	241800	961200	1303000	17.3	132.1
Test item	113.1	-	246000	1010000	1105200	15.1	115.7
Test item	226.3	-	238400	903600	987600	12.5	95.5
Test item	425.5	-	233800	877800	997600	12.5	95.5
Test item	905.0	-	232600	858800	953000	11.7	89.7
Test item	1810.0	-	230400	868000	931400	11.7	89.5
Solvent control w/ DMSO		+	268000	1038000	1598000	20.6	100
Test item	14.1	+	262800	1169800	1518400	22.5	109.2
Test item	28.3		234600	1043800	1287000	19.1	92.5
Test item	56.6		220000	1067400	1154000	18.7	90.5
Test item	113.1		164200	818600	886200	14.7	71.4
Test item	226.3		268200	1029000	1273600	16.3	78.9
Test item	425.5		282800	1255000	1307800	19.3	93.8
Test item	905.0		270200	1184000	723800	10.6	51.2
Test item	1810.0		262000	1146600	940600	13.7	66.5

B. <u>Mutagenicity assay</u> –

Table 2. Summary of Results. Experiment I

	Conc. µg per mL	S9 mix	Relative cloning efficiency 1	Relative total growth	Mutant colonies 10^6 cells	threshold	Relative cloning efficiency 1	Relative total growth	Mutant colonies 10^6 cells	threshold

			Culture I				Culture II			
Solv. Control w/ DMSO		-	100.0	100.0	179	305	100	100	114	240
Pos. control w/MMS	19.5	-	82.1	10.8	1318	305	72.8	34.0	265	240
Test item	56.6	-	108.6	Culture was not continued			100	Culture was not continued		
Test item	113.1	-	86.4	63.0	197	305	85.1	101.6	132	240
Test item	226.3	-	82.1	72.6	195	305	66.2	87.6	143	240
Test item	425.5	-	102.0	87.0	234	305	90.1	123.5	98	240
Test item	905.0	-	100.0	90.2	173	305	76.5	101.5	102	240
Test item	1810.0	-	121.9	75.8	253	305	49.3	80.6	108	240
Solv. Control w/ DMSO		+	100.0	100.0	159	285	100	100.0	171	240
Pos. control w/ CPA	3.0	+	100.0	49.4	230	285	44.8	46.6	157	297
Pos. control w/ CPA	4.5	+	65.9	45.5	350	285	44.8	41.2	376	297
Test item	56.6	+	86.9	Culture was not continued			66.3	Culture was not continued		
Test item	113.1	+	101.6	97.4	161	285	82.0	104.8	154	297
Test item	226.3	+	157.4	111.2	161	285	77.5	91.9	138	297
Test item	425.5	+	80.6	101.6	182	285	78.9	88.9	201	297
Test item	905.0	+	98.4	94.4	211	285	72.1	105.7	146	297
Test item	1810.0	+	110.6	102.3	196	285	77.5	110.1	136	297

Table 3. Summary of results. Experiment II

	Conc. µg per mL	S9 mix	Relative cloning efficiency 1	Relative total growth	Mutant colonies 10^6 cells	threshold	Relative cloning efficiency 1	Relative total growth	Mutant colonies 10^6 cells	threshold
			Culture I				**Culture II**			
Solv. Control w/ DMSO		-	100.0	100.0	166	292	100	100	155	281
Pos. control w/MMS	19.5	-	102.9	54.3	431	292	98.6	49.5	338	281
Test item	56.6	-	119.5	Culture was not continued			107.7	Culture was not continued		
Test item	113.1	-	112.5	50.8	167	292	83.0	88.2	233	281
Test item	226.3	-	155.6	63.9	138	292	90.4	115.2	172	281
Test item	425.5	-	125.1	58.1	186	292	104.5	89.6	197	281
Test item	905.0	-	129.2	58.1	250	292	100	93.7	187	281
Test item	1810.0	-	150.2	37.4	267	292	89.1	105.9	185	281
Solv. Control w/ DMSO		+	100.0	100.0	198	324	100	100.0	148	274
Pos. control w/ CPA	3.0	+	36.4	53.9	238	324	77.3	49.1	199	274
Pos. control w/ CPA	4.5	+	55.5	24.7	505	324	68.0	43.6	394	274
Test item	56.6	+	90.4	Culture was not continued			101.6	Culture was not continued		
Test item	113.1	+	80.7	78.1	171	324	130.5	95.5	156	274
Test item	226.3	+	114.4	69.8	200	324	96.9	64.3	218	274
Test item	425.5	+	107.7	79.6	162	324	82.0	67.5	195	274

Test item	905.0	+	80.7	87.8	171	324	112.4	102.2	151	274
Test item	1810.0	+	78.4	80.9	217	324	148.5	80.1	147	274

No relevant toxic effects indicated by a relative cloning efficiency 1 (survival) or a relative total growth (RTG) of less than 50% in both cultures occurred in experiment I and II up to the maximum concentration with and without metabolic activation. An isolated reduction of the relative cloning efficiency 1 to 49.3 % was noted in the second culture of the first experiment at 1810 µg/mL without metabolic activation. However, the corresponding RTG level was 80.6 % and no cytotoxic effect was observed in the parallel culture under identical conditions. A similar effect occurred in the first culture of the second experiment at 1810 µg/mL. The RTG was reduced to 37.4 % but the corresponding cloning efficiency 1 was 150.2 %. Again, no reduction was noted in both parameters of cytotoxicity in the parallel culture under identical experimental conditions.
No substantial and reproducible dose dependent increase of the mutation frequency was observed in experiment I and II up to the maximum concentration with and without metabolic activation. The threshold of 126 above the corresponding solvent control was not reached or exceeded at any test point with and without metabolic activation.

A single significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in culture I of the second experiment without metabolic activation. Since the mutation frequency did not exceed the threshold in any of the concentrations tested, the statistical result was considered as biologically irrelevant.

In this study the range of the solvent controls was from 114 up to 198 mutant colonies per 10^6 cells; the range of the groups treated with the test item was from 98 up to 267 mutant colonies per 10^6 cells. The solvent controls of the first culture of experiment I without metabolic activation, the second culture of the first experiment with metabolic activation, and the first culture of the second experiment with metabolic activation exceeded the upper limit of the acceptance criteria (170 mutant colonies/10^6 cells). The data are acceptable, since the mutation frequency of each parallel culture remained within the range of 50 – 170 mutant colonies/10^6 cells.

III. Discussion

A. <u>Investigators' conclusions</u> – "In conclusion it can be stated that during the mutagenicity test described and under the experimental conditions reported CSAA798670 did not induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y in the absence and presence of metabolic activation. Therefore, CSAA798670 is considered to be non mutagenic in this mouse lymphoma assay".

B. <u>Reviewer comments</u> - The reviewer agrees with the study author's conclusion. The positive control substances produced the expected response.

C. <u>Study deficiencies</u> – This reviewer found no deficiencies

Report:	IIA 5.1.1/06. Green M & MacDonald M, 2011. SYN545192 - Investigation of the Nature and Identity of Radiolabelled Metabolites Present in Plasma, Urine, Faeces and Bile Collected from Rats Following Oral Administration of [^{14}C]-SYN545192. Charles River, Tranent, Edinburgh, EH32 2NE, UK. Report No. 31096, issue date 31 August 2011. Unpublished (Syngenta File No. SYN545192_10131).EPA MRID No.48604420

Guidelines:

OECD 417 (1984); EPA OPPTS 870.7485 (1998); 87/302/EEC B36 (1987); 94/79/EC (1994)

JMAFF 12 Nohsan No 8147 (2000)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

The toxicokinetic fate of SYN545192 in rats following administration of a single or multiple oral doses was investigated in previous studies [***Shaw, J, 2011a (Syngenta File No. SYN545192_10123); Shaw, J, 2011e (Syngenta File No. SYN545192_10122); Shaw, J, 2011f (Syngenta File No. SYN545192_10120) and Shaw, J, 2011d (Syngenta File No. SYN545192_10121)*]**.** Samples from the toxicokinetic studies were used to investigate the biotransformation of SYN545192 in rats.

The nature and identity of metabolites present in samples of faeces, urine, bile, and plasma following administration of a single oral dose of either 1 mg/kg or 40 mg/kg of [pyrazole-5-^{14}C]-SYN545192 was investigated in male and female rats and bile duct cannulated male and female rats. Biotransformation following administration of 14 consecutive daily oral doses (1 mg/kg) to male rats was also investigated.

Metabolites were identified using a combination of comparative chromatography (combined liquid chromatography and mass spectrometry (LCMS^{n}) and/or thin layer chromatography (TLC)) with standard reference compounds, accurate mass measurement and fragmentation. Selected bile and urine samples were subjected to enzyme hydrolysis using a mixture of β-glucuronidase and sulphatase to assist in the identification of conjugated metabolites. All metabolites accounting for greater than 5% of the administered dose were identified.

SYN545192 was extensively metabolised. The major source of unchanged parent was the faeces with minor amounts found in the plasma and none in the urine or bile, indicating that test substance was rapidly metabolised when it was absorbed. The 8 major types of metabolite consisted of desmethyl, hydroxy, desmethyl hydroxy, ring-open, glucuronide and sulphate conjugates and were mostly conserved through sex and dose, though there were minor differences in quantity and identity.

The major metabolites were SYN546041, SYN546360, SYN546643, SYN546645 and SYN546619, with SYN546039, SYN546042, SYN546708, SYN546644 and glucuronide and sulphate conjugates of the metabolites also identified. Bile and urine samples contained the largest numbers of identified metabolites (19-20 separate metabolites identified), while plasma samples only contained 5 identified metabolites and faecal samples (in cannulated animals) only had parent compound and SYN546039 indicating little to no metabolism via the intestinal flora.

The proposed biotransformation pathway is:

- **Formation of SYN546206 by N-demethylation of SYN545192**
- **Hydroxylation and demethylation to give the major metabolite SYN546041**
- **Hydroxylation of SYN545192 to give the major phenolic metabolite SYN546360**
- **Hydroxylation of both SYN545192 and SYN546206 to give the metabolites SYN546039, SYN546360, SYN546040, SYN546042 and SYN546708**
- **Further hydroxylation to give dihydroxylated metabolites of both SYN545192 and SYN546206 (*e.g.* SYN546619, SYN546644, SYN546645 and SYN546643)**
- **Opening of the bicyclo moiety of both SYN545192 and SYN546206 to give metabolites SYN546634, SYN546706 and SYN546707**
- **Glucuronic acid conjugation and some sulphate conjugation**

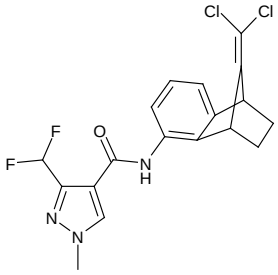
MATERIALS AND METHODS

Materials:

Unlabelled test material:

Name : SYN545192

Structure :



Source : Syngenta Crop Protection Inc.

Physical state : Beige Solid

Batch reference : SMU9BP005

Purity (%w/w) : 97.0

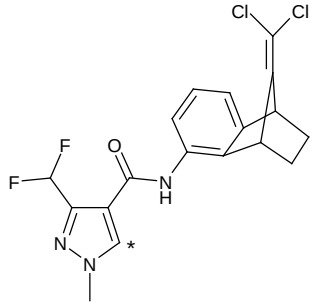
Radiolabelled Test Material: [Pyrazole-5-^{14}C]-SYN545192

Radiochemical purity: >97% (Single 1 mg/kg dose)
>97% (Single 40 mg/kg dose)
>98% (Repeated daily 1 mg/kg dose)

Source: Selcia.

Lot/Batch number: 5072PJS001-2 (Single 1 mg/kg dose)
5072PJS001-2 (Single 40 mg/kg dose)
5120PJS001-1 (Repeated daily 1 mg/kg dose)

Structure:



* position of [^{14}C]-label

Vehicle: The dose vehicle for dose preparations was 1% (w/v) carboxymethylcellulose (CMC) containing 0.1% (v/v) Tween 80.

Preparation of dosing suspensions: Each dose preparation was formulated as a homogenous suspension in 1% CMC in water containing 0.1% Tween 80. The 1 mg/kg dose preparation was

prepared using [^{14}C]-SYN545192 only, the 40 mg/kg dose preparation and the 1 mg/kg dose preparation for the repeat daily dosing study were prepared using [^{14}C]-SYN545192 and non-radiolabelled SYN545192 to achieve the correct dose concentration and specific activity.

Test Animals:

Species:	Rat
Strain:	Han Wistar
Source:	Charles River (UK) Limited

Full details of the test animals were reported in separate studies: [***Shaw, J, 2011a (Syngenta File No. SYN545192_10123); Shaw, J, 2011e (Syngenta File No. SYN545192_10122); Shaw, J, 2011f (Syngenta File No. SYN545192_10120) and Shaw, J, 2011d (Syngenta File No. SYN545192_10121)***].

Study Design and Methods:

Study dates: Start: 31 July 2009; End: 31 August 2011

Origin of Samples: Representative samples of excreta and bile collected following single or multiple oral dosing and selected plasma samples collected from the pharmacokinetic study were pooled for metabolite isolation and quantification as noted in the table below:

Table B.6.1.1/06–1: Origin of samples for metabolite characterisation

Syngenta File Number	Test Group	Position of radiolabel	Dose (mg/kg)
SYN545192_10123	Excretion and tissue distribution	Pyrazole	40
SYN545192_10123	Excretion and tissue distribution	Pyrazole	1
SYN545192_10122	Bile duct cannulation	Pyrazole	40
SYN545192_10122	Bile duct cannulation	Pyrazole	1
SYN545192_10120	Repeat dose study (males only)	Pyrazole	1 mg/kg daily for 14 days
SYN545192_10121	Pharmacokinetic study	Pyrazole	40
SYN545192_10121	Pharmacokinetic study	Pyrazole	1

Dosing and sample collection: In the absorption, distribution and excretion studies, a single oral dose of pyrazole [^{14}C]-SYN545192 formulated as a homogenous suspension in 1% CMC in water containing 0.1% Tween 80 was administered to each rat by gavage in a dose volume of 10 mL/kg corresponding to a dose of 1 or 40 mg/kg. Urine, faeces, and bile as appropriate were collected separately. Urine, bile and faeces were frozen immediately upon collection.

In the repeat dose study, a single daily oral dose of pyrazole [^{14}C]- formulated as a homogenous suspension in 1% CMC in water containing 0.1% Tween 80 was administered to each rat by

gavage in a dose volume of 10 mL/kg corresponding to a dose of 1 mg/kg for 14 consecutive days. Urine and faeces were collected from one group of 3 animals at the 0-24 hour interval after Day 1 and Day 14. Urine and faeces were collected separately and were frozen immediately upon collection.

All excreta samples were analysed for radioactivity by liquid scintillation counting either directly or following sample oxidation.

Further details of dosing and sample collections were reported in separate studies [***Shaw, J, 2011a (Syngenta File No. SYN545192_10123); Shaw, J, 2011e (Syngenta File No. SYN545192_10122); Shaw, J, 2011f (Syngenta File No. SYN545192_10120) and Shaw, J, 2011d (Syngenta File No. SYN545192_10121)***].

Metabolite characterisation studies: Representative samples of urine, faeces and bile from each group were pooled for metabolite identification and quantification.

For the excretion and tissue distribution experiments, urine collected over 0-72 hours for males and 0-96 hours for females and faecal samples collected 0-96 hours for males and 0-120 hours for females were pooled for each dose. For the biliary excretion experiments, urine and faeces samples were pooled to represent 0-48 hour collections and bile samples were pooled to represent 0-48 hour collections for each dose. The representative pooled samples accounted for >95% of the recovered dose. Urine and faeces samples from the repeat dose study were pooled to represent 0-24 hour collections following the first and the fourteenth (final) dose. Plasma samples collected at 6 hours after dosing were pooled to represent metabolite profiles at time points close to T_{max} for each sex at the high dose and 4 hour and 2 hours for the low dose for male and females, respectively.

Urine and bile samples were centrifuged to remove particulates and analysed directly by HPLC-MS^n. Faeces samples were sequentially extracted with acetonitrile, acetonitrile:water (4:1 v/v), acetonitrile:water (1:1 v/v) and acetonitrile:water (1:4 v/v) containing 1% formic acid. With the exception of the acetonitrile:water (1:4 v/v, containing 1% formic acid) extract, all faecal extracts were combined to form a single extract for each sample. The aqueous fraction was reduced to incipient dryness and reconstituted in a suitable volume of acetonitrile:water (1:9 v/v) to facilitate LC-MS^n analysis. Plasma samples were extracted with acetonitrile followed by acetonitrile:water (4:1 v/v), acetonitrile:water (1:1 v/v) and acetonitrile:water (1:4 v/v) containing 1% formic acid. Each acetonitrile extract was reduced to incipient dryness and reconstituted in a suitable volume of acetonitrile:water (1:9 v/v). The acetonitrile:water (1:1 v/v) extract and the acetonitrile:water (1:4 v/v) containing 1% formic acid extract were combined to produce a single acidified aqueous acetonitrile extract for each male rat plasma pool. These were reduced to incipient dryness and reconstituted in a suitable volume of acetonitrile:water (1:9 v/v) to facilitate HPLC and TLC analysis. The acidified aqueous acetonitrile extracts were subjected to glucuronidase and sulphatase enzyme hydrolysis and analysed by TLC.

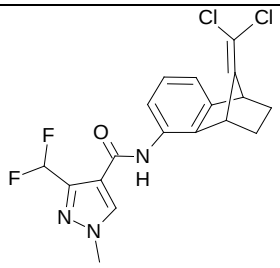
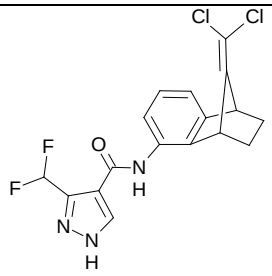
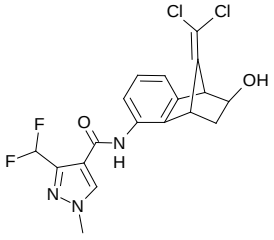
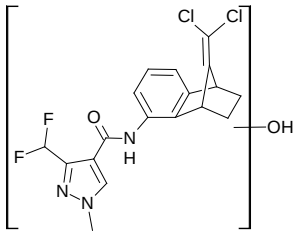
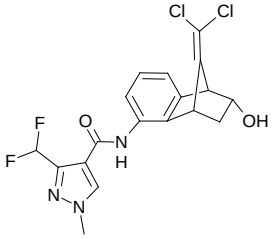
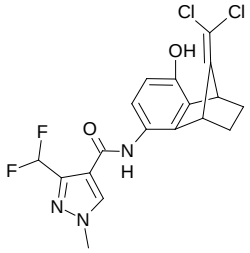
Metabolites were identified by radio-HPLC-MS using a combination of comparative chromatography (LCMS^n and/or TLC) with authentic reference standards, accurate mass measurement and MS^n fragmentation. Selected bile and urine samples were subjected to enzyme hydrolysis using a mixture of β-glucuronidase and sulphatase enzymes to assist in the identification of conjugated metabolites. Selected samples were analysed by TLC. Co-chromatography with reference chemicals was used to confirm the identification of metabolites. Metabolites were quantified by radiochemical detection.

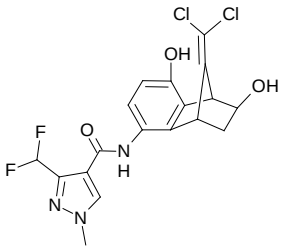
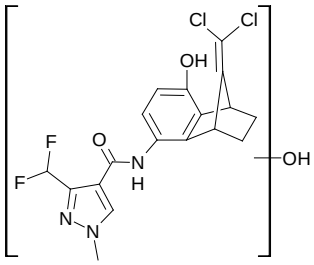
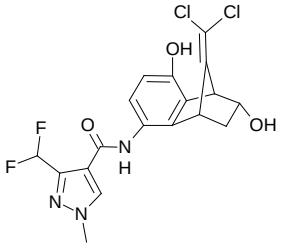
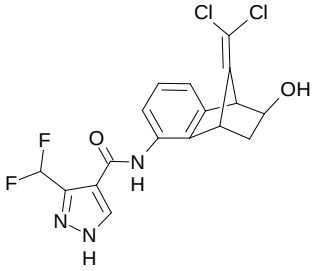
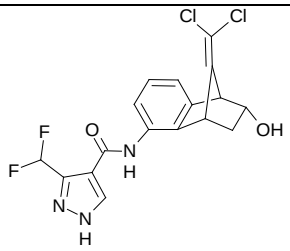
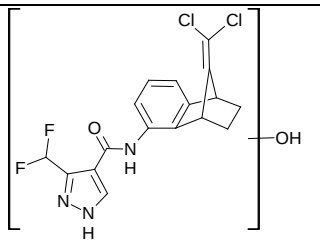
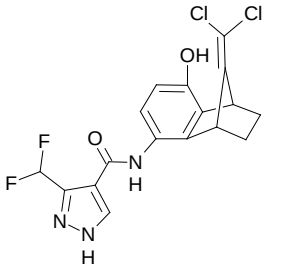
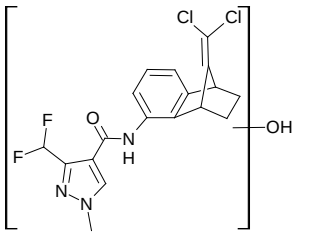
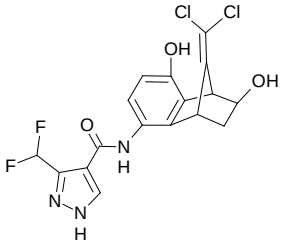
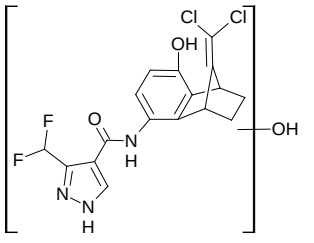
Statistics: Not applicable.

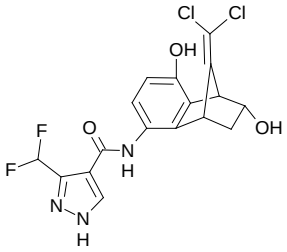
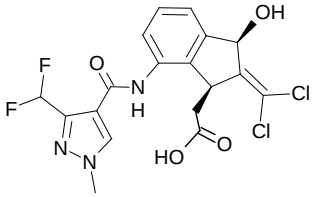
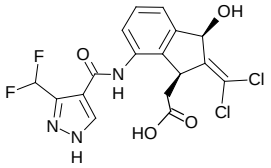
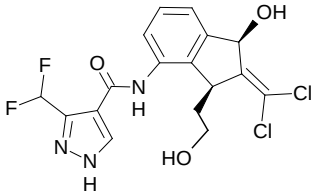
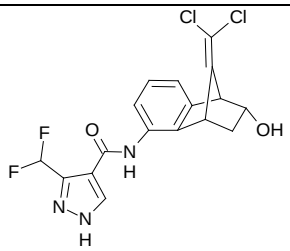
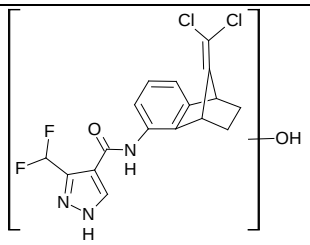
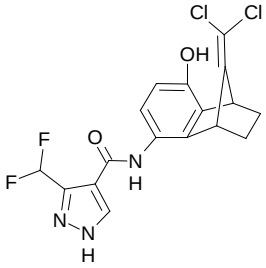
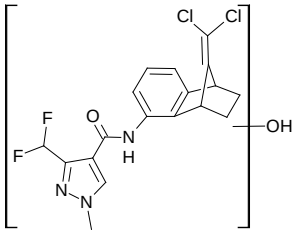
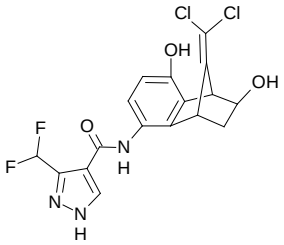
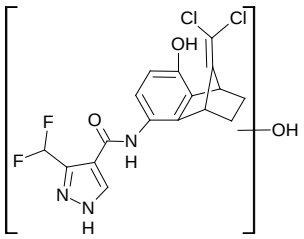
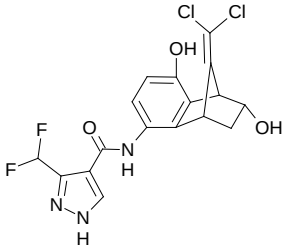
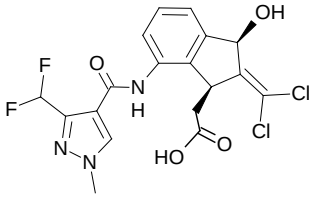
RESULTS AND DISCUSSION

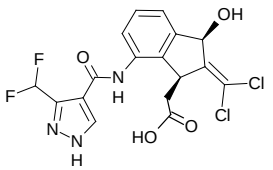
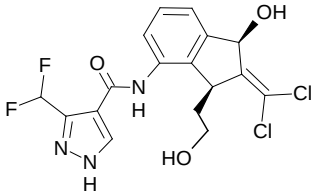
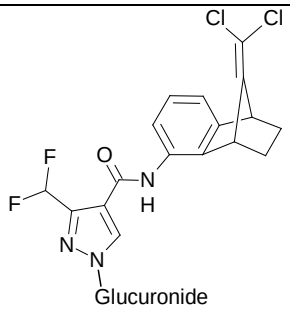
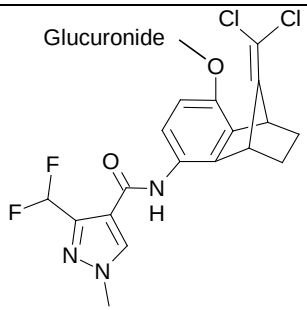
Metabolite characterization studies: Representative pooled samples of urine, faeces and bile were analysed to determine the metabolite profile. The following metabolites of SYN545192 shown in the table below were identified in the rat.

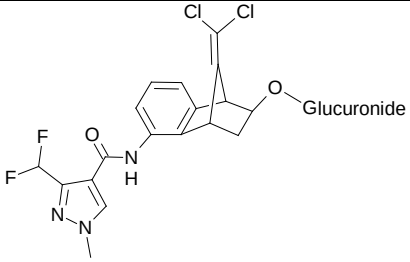
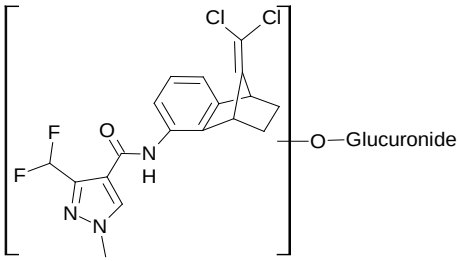
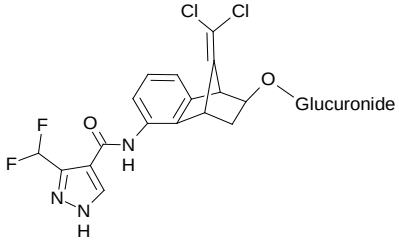
Table B.6.1.1/06–2: Metabolites of [^{14}C]-SYN545192 found in the rat

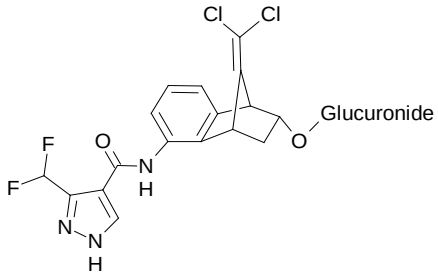
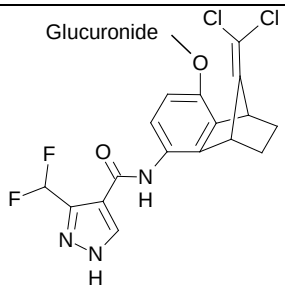
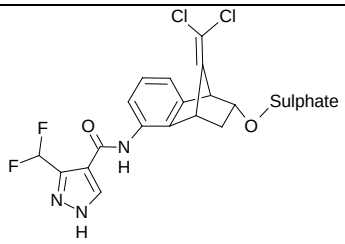
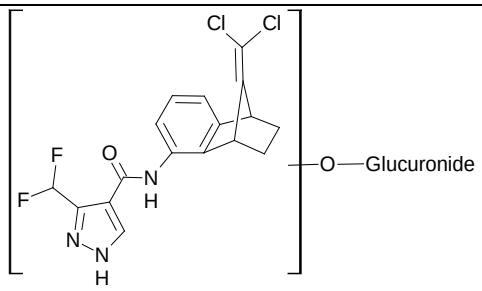
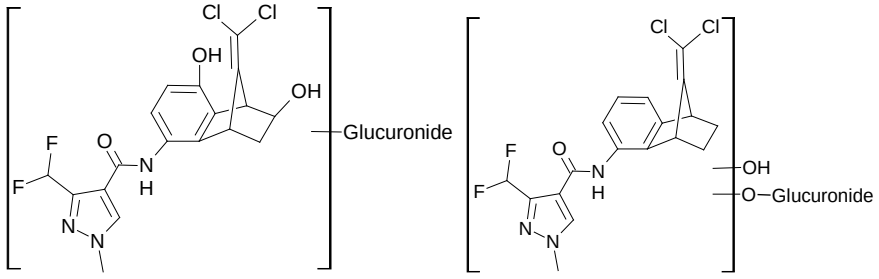
Metabolite name	Structure	Matrix	Metabolite name	Structure	Matrix
SYN545192 (Parent)		Faeces Plasma	SYN546206		Plasma
SYN546039		Plasma Faeces Bile	Hydroxy SYN545192		Bile
SYN546040		Plasma	SYN546360		Urine Faeces Bile

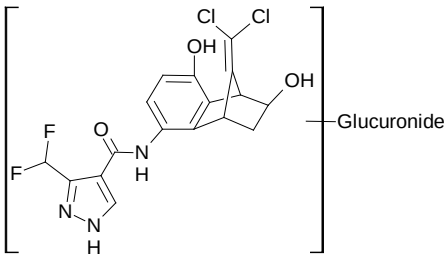
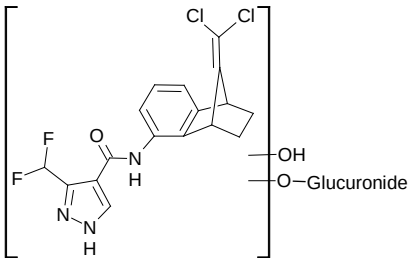
Metabolite name	Structure	Matrix	Metabolite name	Structure	Matrix
SYN546619		Urine Faeces Bile	Dihydroxylated SYN545192		Faeces
SYN546644		Faeces	SYN546041		Urine Plasma Faeces Bile
SYN546042		Urine Plasma Faeces Bile	Hydroxy SYN546206		Bile Urine
SYN546708		Urine Faeces Bile	Hydroxy SYN545192		Bile
SYN546643		Urine Faeces Bile	Dihydroxylated SYN546206		Urine

Metabolite name	Structure	Matrix	Metabolite name	Structure	Matrix
SYN546645		Faeces Bile	SYN546634		Faeces
SYN546706		Urine Faeces	SYN546707		Urine Faeces
SYN546042		Urine Plasma Faeces Bile	Hydroxy SYN546206		Bile Urine
SYN546708		Urine Faeces Bile	Hydroxy SYN545192		Bile
SYN546643		Urine Faeces Bile	Dihydroxylated SYN546206		Urine
SYN546645		Faeces Bile	SYN546634		Faeces

Metabolite name	Structure	Matrix	Metabolite name	Structure	Matrix
SYN546706		Urine Faeces	SYN546707		Urine Faeces
SYN546206 Glucuronide		Bile	SYN546360 Glucuronide		Bile Urine

Metabolite name	Structure	Matrix
SYN546039 Glucuronide		Urine Bile
SYN546643		Bile
SYN546041 Glucuronide		Urine Bile

Metabolite name	Structure	Matrix	Metabolite name	Structure	Matrix
SYN546042 Glucuronide					Urine Bile
Metabolite name	**Structure**	**Matrix**	**Metabolite name**	**Structure**	**Matrix**
SYN546708 Glucuronide		Bile	SYN546042 Sulphate		Urine Faeces
Metabolite name	**Structure**				**Matrix**
Hydroxy SYN546206 Glucuronide					Urine Bile
Dihydroxy Glucuronide Conjugates	 SYN546619 Glucuronide				Urine Bile

Metabolite name	Structure	Matrix	Metabolite name	Structure	Matrix
Dihydroxy SYN546206 Glucuronide	 SYN546643 Glucuronide	Urine Bile			

The metabolite profiles in urine, faeces and bile following administration of a single oral dose of [^{14}C]-SYN545192 at doses of 1 or 40 mg/kg or 14 consecutive daily oral doses of 1 mg/kg are presented in the tables below.

Table B.6.1.1/06–3: Metabolite profile in excreta of rats following a single oral dose of [pyrazole-5-^{14}C]-SYN545192 at the high dose of 40 mg/kg

Compound	Percent of administered dose					
	Male			Female		
	Urine	Faeces	Total excreta	Urine	Faeces	Total excreta
	0-72 h	0-96 h		0-96 h	0-120 h	
SYN545192	ND	0.54	0.54	ND	ND	NA
Not identified	0.83	ND	0.83	0.89	ND	0.89
Not identified	ND	0.70	0.70	ND	ND	NA
Not identified	ND	0.70	0.70	ND	ND	NA
Not identified	ND	1.24	1.24	ND	ND	NA
Not identified	0.55	0.90	1.45	0.51	ND	0.51
Hydroxy SYN546206 glucuronide	ND	ND	NA	0.70	ND	0.70
Not identified	0.46	0.95	1.41	ND	ND	NA
Dihydroxy SYN545192 glucuronide	0.63	ND	0.63	0.79	ND	0.79
SYN546706	0.81	3.65	4.46	ND	ND	NA
SYN546643	0.26	6.20	6.46	ND	4.46	4.46
SYN546634	ND	0.65	0.65	ND	ND	NA
SYN546042 sulphate	ND	ND	NA	0.89	7.08	7.97
SYN546360 glucuronide	0.57	ND	0.57	0.98	ND	0.98
SYN546707	**0.97**	2.48	3.45	ND	ND	NA
SYN546619	ND	4.91	4.91	ND	4.39	4.39
SYN546645	ND	8.02	8.02	ND	1.51	1.51
SYN546041	ND	**33.44**	33.44	0.65	**55.90**	56.55
SYN546644	ND	0.54	0.54	ND	ND	NA
SYN546042	ND	1.38	1.38	ND	2.75	2.75

SYN546039	ND	2.59	2.59	ND	1.25	1.25
SYN546708	0.25	1.04	1.29	ND	ND	NA
SYN546360	0.95	5.73	6.68	**1.20**	2.23	3.43
Additional extracts	NA	1.29	1.29	NA	0.99	0.99
Post extraction solids	NA	7.63	7.63	NA	4.30	4.30
Total identified	4.44	71.17	75.61	5.21	79.57	84.78
Total unidentified	1.84	4.49	6.33	1.40	0.00	1.40
Total accounted for	6.28	84.58	90.86	6.61	84.86	91.47
Losses/Gains	0.01	7.34	7.35	0.00	4.76	4.76
Total	6.29	91.92	98.21	6.61	89.62	96.23
LOQ	0.42	1.16	NA	0.72	1.85	NA

NA = Not applicable, ND = Not detected

Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the % administered dose in each component subtracted from the % administered dose in sample.

LOQ = Limit of quantitation

Table B.6.1.1/06–4: Metabolite profile in excreta of rats following a single oral dose of [pyrazole-5-^{14}C]-SYN545192 at the low dose of 1 mg/kg

Compound	**Percent of administered dose**					
	Male			**Female**		
	Urine	**Faeces**	**Total excreta**	**Urine**	**Faeces**	**Total excreta**
	0-72 h	**0-96 h**		**0-96 h**	**0-120 h**	
SYN545192	ND	0.32	0.32	ND	ND	NA
Not identified	0.34	ND	0.34	ND	ND	NA
Not identified	0.73	ND	0.73	0.53	ND	0.53
Not identified	ND	1.51	1.51	ND	ND	NA
Not identified	ND	1.06	1.06	ND	ND	NA
Not identified	ND	1.07	1.07	ND	ND	NA
Not identified	ND	1.15	1.15	ND	ND	NA
Not identified	0.59	0.87	1.46	0.21	ND	0.21
Not identified	0.41	1.10	1.51	0.67	ND	0.67
Dihydroxy SYN545192 glucuronide	0.58	ND	0.58	0.30	ND	0.30
Not identified	ND	ND	NA	0.35	ND	0.35
Not identified	ND	ND	NA	0.19	ND	0.19
SYN546706	0.83	3.22	4.05	ND	ND	NA
SYN546643	0.40	8.05	8.45	0.18	4.13	4.31
SYN546634	ND	1.07	1.07	ND	ND	NA
SYN546042 sulphate	ND	ND	NA	ND	6.02	6.02
Not identified	ND	ND	NA	0.84	ND	0.84
SYN546360 glucuronide	ND	ND	NA	ND	ND	NA
SYN546707	1.00	3.13	4.13	0.2	ND	0.20
SYN546619	0.66	5.03	5.69	0.39	4.52	4.91
SYN546645	ND	8.39	8.39	ND	1.34	1.34
SYN546041	ND	**25.75**	25.75	0.54	**53.82**	54.36

SYN546644	ND	0.84	0.84	ND	ND	NA
SYN546042	ND	2.10	2.10	0.14	3.62	3.76
SYN546039	ND	0.87	0.87	ND	1.23	1.23
SYN546708	1.14	0.62	1.76	0.21	ND	0.21
SYN546360	**5.16**	4.43	9.59	**1.37**	2.49	3.86
Additional extracts	NA	1.41	1.41	NA	0.99	0.99
Post extraction solids	NA	8.79	8.79	NA	4.85	4.85
Total identified	9.77	63.82	73.59	3.33	77.17	80.50
Total unidentified	2.07	6.76	8.83	2.79	0.00	2.79
Total accounted for	11.84	80.78	92.62	6.12	83.01	89.13
Losses/Gains	-0.01	2.18	2.17	0.01	6.84	6.85
Total	11.83	82.96	94.79	6.13	89.85	95.98
LOQ	0.46	1.83	NA	0.41	1.29	NA

NA = Not applicable, ND = Not detected

Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the % administered dose in each component subtracted from the % administered dose in sample.

LOQ = Limit of quantitation

Table B.6.1.1/06–5: Metabolite profile in excreta of male rats following a repeated oral dosing of [pyrazole-5-^{14}C]-SYN545192 at the low dose of 1 mg/kg

Compound	**Percent of administered dose**					
	0-24 h			**312-336 h**		
	Urine	**Faeces**	**Total excreta**	**Urine**	**Faeces**	**Total excreta**
SYN545192	ND	0.82	0.82	ND	1.61	1.61
Not identified	0.88	-	0.88	0.84	-	0.84
SYN546619 glucuronide	0.45	ND	0.45	0.48	ND	0.48
Not identified	0.27	-	0.27	0.27	-	0.27
Not identified	ND	-	NA	0.25	-	0.25
SYN546708 glucuronide	0.57	ND	0.57	0.49	ND	0.49
SYN546707	0.64	1.43	2.07	0.57	1.91	2.48
Not identified	ND	-	NA	0.34	-	0.34
SYN546360 glucuronide	**2.26**	ND	2.26	**1.45**	ND	1.45
SYN546360	ND	6.26	6.26	0.31	**11.83**	12.14
SYN546706	ND	1.19	1.19	ND	1.60	1.60
SYN546643	ND	3.48	3.48	ND	4.05	4.05
SYN546634	ND	0.86	0.86	ND	0.91	0.91
SYN546619	ND	3.76	3.76	ND	5.22	5.22
SYN546645	ND	5.03	5.03	ND	6.80	6.80
SYN546041	ND	**33.90**	33.90	ND	**38.81**	38.81
SYN546644	ND	0.33	0.33	ND	0.80	0.80
SYN546042	ND	1.35	1.35	ND	2.63	2.63
SYN546039	ND	2.13	2.13	ND	4.59	4.59

SYN546708	ND	0.94	0.94	ND	2.44	2.44
Additional extracts	ND	0.65	0.65	ND	0.56	0.56
Post extraction solids	ND	4.68	4.68	ND	6.43	6.43
Total identified	3.92	61.48	65.40	3.30	83.20	86.50
Total unidentified	1.15	0.00	1.15	1.70	0.00	1.70
Total accounted for	5.07	66.81	71.88	5.00	90.19	95.19
Losses/Gains	0.00	5.14	5.14	-0.02	2.96	2.94
Total	5.07	71.95	77.02	4.98	93.15	98.13
LOQ	0.20	1.64	NA	0.17	1.25	NA

NA = Not applicable, ND = Not detected

Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the % administered dose in each component subtracted from the % administered dose in sample.

LOQ = Limit of quantitation

Table B.6.1.1/06–6: Summary of Metabolites in Excreta from Rats Following a Single Oral Dose of 40 mg [^{14}C]-SYN545192/kg Subject to Enzyme Hydrolysis

Compound	Percent of administered dose					
	Male			Female		
	Urine	Faeces	Total excreta	Urine	Faeces	Total excreta
	0-72 h	0-96 h		0-96 h	0-120 h	
SYN545192	ND	0.54	0.54	ND	ND	NA
Not identified	1.31	-	1.31	1.57	-	1.57
SYN546619 glucuronide	0.51	ND	0.51	ND	ND	NA
Not identified	-	0.70	0.70	-	ND	NA
Not identified	-	0.70	0.70	-	ND	NA
Not identified	-	1.24	1.24	-	ND	NA
Not identified	-	0.90	0.90	-	ND	NA
Not identified	-	0.95	0.95	-	ND	NA
SYN546706	ND	3.65	3.65	ND	ND	NA
SYN546643	0.76	6.20	6.96	0.55	4.46	5.01
SYN546634	ND	0.65	0.65	ND	ND	NA
SYN546042 sulphate	ND	ND	NA	ND	7.08	7.08
SYN546707	ND	2.48	2.48	ND	ND	NA
SYN546619	**2.19**	4.91	7.10	1.42	4.39	5.81
SYN546645	ND	8.02	8.02	ND	1.51	1.51
SYN546041	ND	**33.44**	33.44	**1.81**	**55.90**	57.71
SYN546644	ND	0.54	0.54	ND	ND	NA
SYN546042	ND	1.38	1.38	ND	2.75	2.75
SYN546039	ND	2.59	2.59	ND	1.25	1.25
SYN546708	ND	1.04	1.04	ND	ND	NA
SYN546360	1.52	5.73	7.25	1.26	2.23	3.49
Additional extracts	NA	1.29	1.29	NA	0.99	0.99

Post extraction solids	NA	7.63	7.63	NA	4.30	4.30
Total identified	4.98	71.17	76.15	5.04	79.57	84.61
Total unidentified	1.31	4.49	5.80	1.57	0.00	1.57
Total accounted for	6.29	84.58	90.87	6.61	84.86	91.47
Losses/Gains	0.00	7.34	7.34	0.00	4.76	4.76
Total	6.29	91.92	98.21	6.61	89.62	96.23
LOQ	0.28	1.16	NA	0.48	1.85	NA

NA = Not applicable, ND = Not detected

Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the % administered dose in each component subtracted from the % administered dose in sample.

LOQ = Limit of quantitation

Table B.6.1.1/06–7: Summary of Metabolites in Excreta from Rats Following a Single Oral Dose of 1 mg [^{14}C]-SYN545192/kg Subject to Enzyme Hydrolysis

Compound	Percent of administered dose					
	Male			Female		
	Urine 0-72 h	Faeces 0-96 h	Total excreta	Urine 0-96 h	Faeces 0-120 h	Total excreta
SYN545192	ND	0.32	0.32	ND	ND	NA
Not identified	1.32	-	1.32	0.78	-	0.78
SYN546619 glucuronide	0.31	ND	0.31	ND	ND	NA
Not identified	-	1.51	1.51	-	ND	NA
Not identified	-	1.06	1.06	-	ND	NA
Not identified	-	1.07	1.07	-	ND	NA
Not identified	-	1.15	1.15	-	ND	NA
Not identified	-	0.87	0.87	-	ND	NA
Not identified	-	1.10	1.10	-	ND	NA
SYN546706	ND	3.22	3.22	ND	ND	NA
SYN546643	0.53	8.05	8.58	ND	4.13	4.13
SYN546634	ND	1.07	1.07	ND	ND	NA
SYN546042 sulphate	ND	ND	NA	ND	6.02	6.02
SYN546707	ND	3.13	3.13	ND	ND	NA
SYN546619	3.47	5.03	8.50	1.78	4.52	6.30
Not identified	0.58	-	0.58	ND	ND	NA
SYN546645	ND	8.39	8.39	ND	1.34	1.34
SYN546041	ND	**25.75**	25.75	1.75	**53.82**	55.57
SYN546644	ND	0.84	0.84	ND	ND	NA
SYN546042	ND	2.10	2.10	ND	3.62	3.62
SYN546039	ND	0.87	0.87	ND	1.23	1.23
SYN546708	0.99	0.62	1.61	ND	ND	NA
SYN546360	**4.62**	4.43	9.05	**1.82**	2.49	4.31
Additional extracts	NA	1.41	1.41	NA	0.99	0.99

Post extraction solids	NA	8.79	8.79	NA	4.85	4.85
Total identified	9.92	63.82	73.74	5.35	77.17	82.52
Total unidentified	1.90	6.76	8.66	0.78	0.00	0.78
Total accounted for	11.82	80.78	92.60	6.13	83.01	89.14
Losses/Gains	0.01	2.18	2.19	0.00	6.84	6.84
Total	11.83	82.96	94.79	6.13	89.85	95.98
LOQ	0.46	1.83	NA	0.54	1.29	NA

NA = Not applicable, ND = Not detected

Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the % administered dose in each component subtracted from the % administered dose in sample.

LOQ = Limit of quantitation

Table B.6.1.1/06–8: Summary of Metabolites in Excreta from Bile Duct Cannulated Rats Following a Single Oral Dose of 40 mg [^{14}C]-SYN545192/kg

Compound	Percent of administered dose							
	Male				Female			
	Urine	Faeces	Bile	Total excreta	Urine	Faeces	Bile	Total excreta
	0-48 h	0-48 h	0-48 h		0-48 h	0-48 h	0-48 h	
SYN545192	ND	**19.91**	ND	19.91	ND	**25.60**	ND	25.60
Not identified	0.11	ND	ND	0.11	ND	ND	ND	NA
Not identified	0.68	ND	ND	0.68	0.45	ND	ND	0.45
Not identified	0.25	ND	ND	0.25	ND	ND	ND	NA
Hydroxy SYN545192 glucuronide	ND	ND	0.72	0.72	ND	ND	ND	NA
Not identified	0.14	ND	ND	0.14	ND	ND	ND	NA
SYN546643 glucuronide	ND	ND	0.57	0.57	ND	ND	ND	NA
Dihydroxy SYN546206 glucuronide	0.41	ND	1.31	1.72	0.63	ND	0.97	1.60
Dihydroxy SYN546206 glucuronide	ND	ND	ND	NA	ND	ND	0.63	0.63
SYN546619 glucuronide	0.96	ND	2.02	2.98	0.70	ND	0.94	1.64
SYN546643	0.38	ND	ND	0.38	0.22	ND	ND	0.22
SYN546041 glucuronide	2.66	ND	**35.32**	37.98	**6.79**	ND	**25.22**	32.01
SYN546042 glucuronide	ND	ND	1.41	1.41	0.39	ND	2.31	2.70
Dihydroxy SYN546206	0.14	ND	ND	0.14	ND	ND	ND	NA
Hydroxy SYN545192 glucuronide	ND	ND	1.90	1.90	ND	ND	1.09	1.09
SYN546708 glucuronide	**1.12**	ND	4.71	5.83	0.65	ND	0.79	4.12
SYN546039 glucuronide	(a)	ND	(a)	(b)	(a)	ND	2.68	(b)
SYN546619	0.58	ND	ND	0.58	0.57	ND	ND	0.57
SYN546707	(a)	ND	ND	(b)	ND	ND	ND	NA
SYN546706	0.28	ND	ND	0.28	ND	ND	ND	NA
SYN546360 glucuronide	0.65	ND	7.17	7.82	0.30	ND	4.59	4.89
SYN546041	0.21	ND	ND	0.21	0.50	ND	1.01	1.51

Dihydroxy SYN546206	ND	ND	ND	NA	0.21	ND	ND	0.21
SYN546039	ND	2.37	ND	2.37	ND	1.25	ND	1.25
SYN546708	0.22	ND	ND	0.22	0.31	ND	ND	0.31
SYN546360	0.93	ND	ND	0.93	2.54	ND	1.13 (c)	2.54
SYN546042 sulphate	ND	ND	ND	NA	(a)	ND	ND	NA
Hydroxy SYN545192 glucuronide	ND	ND	ND	NA	ND	ND	0.99	0.99
Hydroxy SYN546206 glucuronide	ND	ND	ND	NA	ND	ND	(c)	(c)
Not identified	0.27	ND	ND	0.27	ND	ND	ND	ND
SYN546206 glucuronide	ND	ND	ND	NA	ND	ND	0.95	0.95
Hydroxy SYN546206	ND	ND	ND	NA	0.18	ND	1.35	1.53
Post extraction solids	NA	0.49	NA	0.49	NA	0.35	NA	0.35
Total identified	8.54	22.28	55.13	85.95	13.99	26.85	44.65	85.49
Total unidentified	1.45	0.00	0.00	1.45	0.45	0.00	0.00	0.45
Total accounted for	9.99	22.77	55.13	87.89	14.44	27.20	44.65	86.29
Losses/Gains	0.00	4.27	-0.01	4.26	0.01	4.73	0.01	4.75
Total	9.99	27.04	55.12	92.15	14.45	31.93	44.66	91.04
LOQ	0.12	0.88	0.88	NA	0.22	0.48	1.01	NA

(a) Unresolved components

(b) Total including unresolved components

(c) SYN546360 and hydroxy SYN546206 glucuronide present as unresolved components accounting for a total of 1.13%

NA = Not applicable, ND = Not detected

Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the % administered dose in each component subtracted from the % administered dose in sample.

LOQ = Limit of quantitation

Table B.6.1.1/06–9: Summary of Metabolites in Excreta from Bile Duct Cannulated Rats Following a Single Oral Dose of 1 mg [^{14}C]-SYN545192/kg

Compound	Percent of administered dose							
	Male				Female			
	Urine 0-48 h	Faeces 0-48 h	Bile 0-48 h	Total excreta	Urine 0-48 h	Faeces 0-48 h	Bile 0-48 h	Total excreta
SYN545192	ND	**14.29**	ND	14.29	ND	**12.65**	ND	12.65
Not identified	0.31	ND	ND	0.31	0.75	ND	ND	0.75
Not identified	0.12	ND	ND	0.12	ND	ND	ND	NA
SYN546619 glucuronide	0.42	ND	ND	0.42	0.33	ND	ND	0.33
SYN546643	0.08	ND	1.96	2.04	ND	ND	1.62	1.62
SYN546041 glucuronide	**0.88**	ND	ND	0.88	**1.59**	ND	ND	1.59
Dihydroxy SYN546206	0.15	ND	ND	0.15	ND	ND	ND	NA
Not identified	ND	ND	ND	NA	0.19	ND	ND	0.19
SYN546708 glucuronide	0.63	ND	ND	0.63	0.33	ND	ND	0.33
SYN546039 glucuronide	(a)	ND	ND	(a)	(a)	ND	ND	(a)
SYN546619	0.21	ND	2.05	2.26	ND	ND	2.99	2.99
SYN546707	(a)	ND	ND	(a)	ND	ND	ND	NA

SYN546645	ND	ND	1.16	1.16	ND	ND	ND	NA
Not identified	0.05	ND	ND	0.05	ND	ND	ND	NA
SYN546706	0.05	ND	ND	0.05	ND	ND	ND	NA
SYN546360 glucuronide	0.33	ND	ND	0.33	0.30	ND	ND	0.30
SYN546041	0.17	ND	**44.25**	44.42	0.16	ND	**45.25**	45.41
SYN546042	ND	ND	2.15	2.15	ND	ND	1.62	1.62
SYN546039	ND	1.55	14.61	16.16	ND	1.63	10.05	11.68
SYN546708	0.03	ND	1.29	1.32	0.08	ND	1.05	1.13
SYN546360	0.16	ND	6.91	7.07	0.26	ND	4.72	4.98
Hydroxy SYN545192	ND	ND	ND	NA	ND	ND	1.17	1.17
Not identified	ND	ND	1.69	1.69	ND	ND	ND	NA
Post extraction solids	NA	0.47	NA	0.47	NA	0.36	NA	0.36
Total identified	3.11	15.84	74.38	93.33	3.05	14.28	68.47	85.80
Total unidentified	0.48	0.00	1.69	2.17	0.94	0.00	0.00	0.94
Total accounted for	3.59	16.31	76.07	95.97	3.99	14.64	68.47	87.10
Losses/Gains	0.00	0.58	0.00	0.58	0.00	1.17	-0.01	1.16
Total	3.59	16.89	76.07	96.55	3.99	15.81	68.46	88.26
LOQ	0.10	2.80	1.65	NA	0.34	2.78	1.22	NA

Note: Urine and faecal extracts were not hydrolysed

(a) Unresolved components

NA = Not applicable, ND = Not detected

Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the % administered dose in each component subtracted from the % administered dose in sample.

LOQ = Limit of quantitation

Table B.6.1.1/06–10: Summary of Metabolites in Excreta from Bile Duct Cannulated Rats Following a Single Oral Dose of 40 mg [^{14}C]-SYN545192/kg Subject to Enzyme Hydrolysis

Compound	Percent of administered dose							
	Male				Female			
	Urine 0-48 h	Faeces 0-48 h	Bile 0-48 h	Total excreta	Urine 0-48 h	Faeces 0-48 h	Bile 0-48 h	Total excreta
SYN545192	ND	**19.91**	ND	19.91	ND	**25.60**	ND	25.60
Not identified	0.11	ND	ND	0.11	ND	ND	ND	NA
Not identified	0.68	ND	ND	0.68	0.45	ND	ND	0.45
Not identified	0.25	ND	ND	0.25	ND	ND	ND	NA
Not identified	0.14	ND	ND	0.14	ND	ND	ND	NA
Dihydroxy SYN546206 glucuronide	0.41	ND	ND	0.41	0.63	ND	ND	0.63
SYN546619 glucuronide	0.96	ND	ND	0.96	0.70	ND	ND	0.70
SYN546643	0.38	ND	2.00	2.38	0.22	ND	0.66	0.88
SYN546041 glucuronide	**2.66**	ND	ND	2.66	**6.79**	ND	ND	6.79
SYN546042 glucuronide	ND	ND	ND	NA	0.39	ND	ND	0.39
Dihydroxy SYN546206	0.14	ND	ND	0.14	ND	ND	ND	NA

SYN546708 glucuronide	1.12	ND	ND	1.12	0.65	ND	ND	0.65
SYN546039 glucuronide	(a)	ND	ND	(a)	(a)	ND	ND	(a)
SYN546619	0.58	ND	3.00	3.58	0.57	ND	1.21	1.78
SYN546707	(a)	ND	ND	(b)	ND	ND	ND	NA
SYN546645	ND	ND	1.05	1.05	ND	ND	0.89	0.89
SYN546706	0.28	ND	ND	0.28	ND	ND	ND	NA
SYN546360 glucuronide	0.65	ND	ND	0.65	0.30	ND	ND	0.30
SYN546041	0.21	ND	**34.85**	35.06	0.50	ND	**28.70**	29.20
Dihydroxy SYN546206	ND	ND	ND	NA	0.21	ND	ND	0.21
SYN546042	ND	ND	0.77	0.77	ND	ND	2.34	2.34
SYN546039	ND	2.37	4.63	7.00	ND	1.25	3.60	4.85
SYN546708	0.22	ND	1.72	1.94	0.31	ND	1.13	1.44
SYN546360	0.93	ND	5.74	6.67	2.54	ND	5.36	7.90
SYN546206 sulphate	ND	ND	ND	NA	(a)	ND	ND	(b)
Not identified	0.27	ND	ND	0.27	ND	ND	ND	NA
Not identified	ND	ND	1.37	1.37	ND	ND	ND	NA
Hydroxy SYN545192	ND	ND	ND	NA	ND	ND	0.78	0.78
Hydroxy SYN546206	ND	ND	ND	NA	0.18	ND	ND	0.18
Post extraction solids	NA	0.49	NA	0.49	NA	0.35	NA	0.35
Total identified	8.54	22.28	53.76	84.58	13.99	26.85	44.67	85.51
Total unidentified	1.45	0.00	1.37	2.82	0.45	0.00	0.00	0.45
Total accounted for	9.99	22.77	55.13	87.89	14.44	27.20	44.67	86.31
Losses/Gains	0.00	4.27	-0.01	4.26	0.01	4.73	-0.01	4.73
Total	9.99	27.04	55.12	92.15	14.45	31.93	44.66	91.04
LOQ	0.12	0.88	0.88	NA	0.22	0.48	1.01	NA

Note: Urine and faecal extracts were not hydrolysed

(a) Unresolved components

(b) Total included unresolved components

NA = Not applicable, ND = Not detected

Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the % administered dose in each component subtracted from the % administered dose in sample.

LOQ = Limit of quantitation

Table B.6.1.1/06–11: Summary of Metabolites in Excreta from Bile Duct Cannulated Rats Following a Single Oral Dose of 1 mg [^{14}C]-SYN545192/kg Subject to Enzyme Hydrolysis

Compound	Percent of administered dose							
	Male				Female			
	Urine 0-48 h	Faeces 0-48 h	Bile 0-48 h	Total excreta	Urine 0-48 h	Faeces 0-48 h	Bile 0-48 h	Total excreta
SYN545192	ND	**14.29**	ND	14.29	ND	**12.65**	ND	12.65
Not identified	0.31	ND	ND	0.31	0.75	ND	ND	0.75
Not identified	0.12	ND	ND	0.12	ND	ND	ND	NA
SYN546619 glucuronide	0.42	ND	ND	0.42	0.33	ND	ND	0.33

SYN546643	0.08	ND	1.96	2.04	ND	ND	1.62	1.62
SYN546041 glucuronide	**0.88**	ND	ND	0.88	**1.59**	ND	ND	1.59
Dihydroxy SYN546206	0.15	ND	ND	0.15	ND	ND	ND	NA
Not identified	ND	ND	ND	NA	0.19	ND	ND	0.19
SYN546708 glucuronide	0.63	ND	ND	0.63	0.33	ND	ND	0.33
SYN546039 glucuronide	(a)	ND	ND	(a)	(a)	ND	ND	(a)
SYN546619	0.21	ND	2.05	2.26	ND	ND	2.99	2.99
SYN546707	(a)	ND	ND	(a)	ND	ND	ND	NA
SYN546645	ND	ND	1.16	1.16	ND	ND	ND	NA
Not identified	0.05	ND	ND	0.05	ND	ND	ND	NA
SYN546706	0.05	ND	ND	0.05	ND	ND	ND	NA
SYN546360 glucuronide	0.33	ND	ND	0.33	0.30	ND	ND	0.30
SYN546041	0.17	ND	**44.25**	44.42	0.16	ND	**45.25**	45.41
SYN546042	ND	ND	2.15	2.15	ND	ND	1.62	1.62
SYN546039	ND	1.55	14.61	16.16	ND	1.63	10.05	11.68
SYN546708	0.03	ND	1.29	1.32	0.08	ND	1.05	1.13
SYN546360	0.16	ND	6.91	7.07	0.26	ND	4.72	4.98
Hydroxy SYN545192	ND	ND	ND	NA	ND	ND	1.17	1.17
Not identified	ND	ND	1.69	1.69	ND	ND	ND	NA
Post extraction solids	NA	0.47	NA	0.47	NA	0.36	NA	0.36
Total identified	3.11	15.84	74.38	93.33	3.05	14.28	68.47	85.80
Total unidentified	0.48	0.00	1.69	2.17	0.94	0.00	0.00	0.94
Total accounted for	3.59	16.31	76.07	95.97	3.99	14.64	68.47	87.10
Losses/Gains	0.00	0.58	0.00	0.58	0.00	1.17	-0.01	1.16
Total	3.59	16.89	76.07	96.55	3.99	15.81	68.46	88.26
LOQ	0.10	2.80	1.65	NA	0.34	2.78	1.22	NA

Note: Urine and faecal extracts were not hydrolysed

(a) Unresolved components

NA = Not applicable, ND = Not detected

Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the % administered dose in each component subtracted from the % administered dose in sample.

LOQ = Limit of quantitation

Table B.6.1.1/06–12: Summary of Metabolites in Plasma from Bile Duct Cannulated Rats Following a Single Oral Dose of 1 mg [^{14}C]-SYN545192/kg Subject to Enzyme Hydrolysis

Compound	Percent of administered dose			
	Male		Female	
	40 mg/kg bw	1 mg/kg bw	40 mg/kg bw	1 mg/kg bw
	6 h	4 h	6 h	2 h
SYN545192	0.335	0.013	0.338	0.040
SYN546040	0.033	0.002	ND	ND
Not identified	ND	0.001	ND	ND
SYN546039	0.247	0.012	0.070	0.013

SYN546206	0.046	0.001	**0.648**	**0.043**
Not identified	ND	0.001	ND	ND
Not identified	0.054	ND	ND	ND
SYN546041	**0.486**	**0.023**	0.340	0.028
Not identified	0.008	0.001	ND	ND
Not identified	0.138	0.006	ND	ND
Not identified	0.042	0.002	ND	ND
Origin material	0.327	0.018	0.131	0.012
Additional extracts	0.834	0.093	NA	NA
Post extraction solids	0.997	0.058	0.068	0.005
Total identified	1.344	0.062	1.489	0.144
Total unidentified (a)	0.569	0.029	0.131	0.012
Total accounted for	3.744	0.242	1.688	0.161
Losses/Gains	0.443	0.006	0.064	-0.011
Total	4.187	0.248	1.752	0.150
LOQ	0.008	0.001	0.070	0.012

(a) Including origin material

NA = Not applicable, ND = Not detected

Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the % administered dose in each component subtracted from the % administered dose in sample.

LOQ = Limit of quantitation

SYN545192 metabolites in faeces: In the bile cannulation studies, there were only two faecal metabolites, unchanged parent (comprising 12 – 26% of the administered dose) and the desmethyl hydroxyl metabolite SYN546039 (comprising 1 – 3% of the administered dose). The unchanged parent comprised 14.29 and 12.65% of the administered dose in males and females, respectively, in the low-dose and 19.91 and 25.6% of the administered dose in high-dose males and females, respectively.

In the non-cannulated studies, the major metabolite is SYN546041 in all samples and occurred in the faeces, urine, bile and plasma. Other metabolites were mostly conserved throughout the non-cannulated faecal samples. There were changes in quantity and identity of some of the less prevalent metabolites. Ring-open metabolites were detected in faeces from males only while a sulphate conjugate of SYN546042 was present in faeces from females only.

Metabolites that occur in all of the non-bile cannulated faecal samples are SYN546039, 546041, 646042, 646360, 546619, 545543 and 546645. SYN545192, 546634, 546644, 546706, 546707 and 546708 occurred in all male samples, but only in the female repeat-dose sample. SYN546042 sulphate occurred in female single oral low- and high-dose samples, but not in the female repeat-dose sample.

SYN545192 metabolites in urine: Urinary metabolites comprised only 3.59-14.45% of the administered dose, indicating urine was a minor excretory route. The major metabolites were

conserved between doses and sexes and no parent compound was detected. Many of the metabolites occurred as both a compound and its glucuronide or sulphate. SYN546206 appeared as both a hydroxy and dihydroxy. The largest differences between the samples were between the bile cannulated samples and the non-cannulated samples in that bile-cannulated samples contained SYN546041 glucuronide, no 546042 and do contain 546708 glucuronide, which otherwise only appears in repeat-dose samples. SYN546041 occurred in all cannulated males, but only in females in non-cannulated samples and dihydroxy SYN545192 glucuronide occurred in only in single oral dose samples not subject to enzyme hydrolysis while the SYN546206 dihydroxy, hydroxy and dihydroxy glucuronide appeared in cannulated samples, but hydroxyl SYN546206 glucuronide only occurred in female 40 mg/kg non-cannulated samples not subject to enzyme hydrolysis. Two metabolites in occurred in males and females in the bile, but, in the urine, SYN546039 and 546645 only occurred in 40 mg/kg female urine samples subjected to enzyme hydrolysis.

Up to 25 metabolites were detected on radiochromatograms for urine samples following oral administration of [^{14}C]-SYN545192 to rats and 20 of those were identified. SYN545192 was not detected in any urine sample. Identified compounds accounted for 3 – 14% of the administered dose and the most abundant was SYN546360 which accounted for 0.31-5.16% dose.

SYN545192 metabolites in bile: The major metabolites in the bile were SYN546041 or SYN546041 glucuronide. High-dose males had SYN546041 glucuronide in the bile sample in the absence of SYN546041 and high- and low-dose females had SYN546041 as a minor metabolite in the presence of SYN546041 glucuronide as the major metabolite. In samples subjected to enzyme hydrolysis, in low-dose males, SYN546041 was the major metabolite and there was no evidence of SYN546041 glucuronide. Bile metabolites accounted for 45 – 76% of the administered dose and only three metabolites (2 in low-dose female samples and one in the high-dose male sample subjected to enzyme hydrolysis) were unidentified.

In the enzyme hydrolysed samples, the only sex differences were an unidentified metabolite in 40 mg/kg bw male samples, a lack of SYN546645 in low-dose female samples and the presence of hydroxyl SYN545192 only in the female samples.

Up to 19 components were detected in radiochromatograms for bile samples following oral administration of [^{14}C]-SYN545192 to rats. The 18 components that were identified accounted for 44.65-75.05% of the dose in total. SYN545192 was not detected in any bile sample. Up to 10 components were detected in the radiochromatograms for hydrolysed bile samples. The 9 identified components accounted for 44.67-74.38% dose in total and were consistent with the conjugated metabolites identified in unhydrolysed bile.

SYN545192 metabolites in plasma: Plasma was a relatively minor reservoir for metabolites containing < 5% of the administered dose. Parent compound, SYN545192, accounted for 0.34% of the administered dose in animals given 40 mg/kg bw and 0.01 and 0.04% in males and

females given 1 mg/kg bw, respectively. The major metabolite in males was SYN546041 and SYN546206 in females. Of the 11 isolated metabolites, five were identified in both males and females and only SYN546040 appeared in males only. Of the six unidentified metabolites, all were only found in males and one each was only found in either high or low-dose males.

Overall SYN545192 was extensively metabolised by rats giving rise to at least 8 types of metabolite (*e.g.* desmethyl, hydroxy, dihydroxy, desmethyl hydroxy, desmethyl dihydroxy, ring-open, glucuronide conjugate, sulphate conjugate). The major metabolites were SYN546041, SYN546360, SYN546039, SYN546643 and SYN546645. Other identified components included SYN546206, SYN546042, SYN546619, SYN546644 and SYN546708. Glucuronide and, in some cases, sulphate conjugates of these metabolites were also present. Ring open metabolites SYN546634, SYN546706 and SYN546707 were detected primarily in males.

Metabolic pathway:

The biotransformation proceeded by:

- Formation of SYN546206 by N-demethylation of SYN545192
- Hydroxylation of SYN546206 to give the major metabolite SYN546041
- Hydroxylation of both SYN545192 and SYN546206 to give the metabolites SYN546039, SYN546360, SYN546040 SYN546042, SYN546708
- Further hydroxylation to give dihydroxylated metabolites of both SYN545192 and SYN546206 (*e.g.* SYN546619, SYN546644, SYN546645 and SYN546643)
- Opening of the bicyclo moiety of both SYN545192 and SYN546206 to give the ring open metabolites SYN546634, SYN546706 and SYN546707
- Glucuronic acid conjugation and some sulphate conjugation

The biotransformation pathway proposed for SYN545192 is presented in the Figure B.6.1.1/06–1.

INVESTIGATORS' CONCLUSION: This study has shown that SYN545192 was extensively metabolised in rat giving rise to at least 8 types of metabolite (*e.g.* desmethyl, hydroxy, desmethyl hydroxy, ring-open, glucuronide and sulphate conjugates). The majority, between 70 and 85%, of the administered radioactivity was identified following a single oral dose of SYN545192 to rats. The major metabolites were identified as SYN546041, SYN546360, SYN546643, SYN546645 and SYN546619. Other identified components included SYN546039, SYN546042, SYN546708 and SYN546644. Glucuronide and, in some cases sulphate, conjugates of these metabolites were also present. While some quantitative differences were observed between males and females, SYN546041 and SYN546360 together accounted for the majority of the dose (35-60% dose). No significant differences were observed following a single

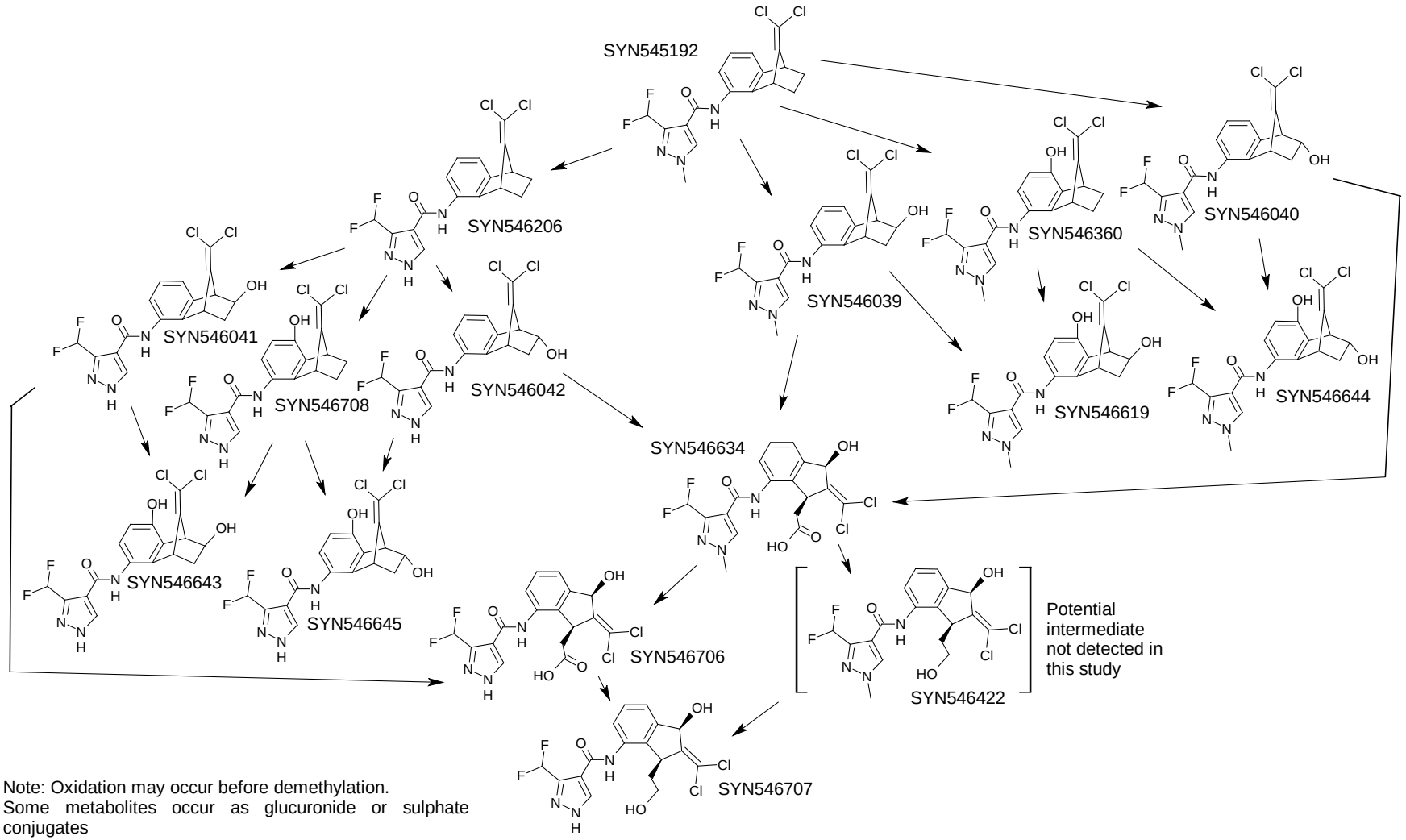
dose at 40 mg/kg or 1 mg/kg or repeat daily dosing at 1 mg/kg/day. Minor differences between males and females were indicated by the presence of ring-open metabolites in males primarily and the presence of the sulphate conjugate of SYN546042 in females only. There was little evidence to indicate cleavage of SYN545192 between the pyrazole and phenyl moieties with possible metabolites present at <2% dose in urine only.

Reviewer's Conclusions: The reviewer agrees with the investigator's conclusions. SYN545192 is extensively metabolised in males and females and in low and high doses. Parent compound was only found in the faeces and in small amounts in the plasma, indicating that whatever compound is absorbed is quickly metabolised.

The major metabolites were SYN546041, SYN546360, SYN546643, SYN546645 and SYN546619, with desmethyl, hydroxy, desmethyl hydroxy, ring-open, glucuronide and sulphate conjugates occurring commonly.

There were minor differences in quantity and identity of metabolites between males and females and high and low doses; however, most metabolites were conserved.

Figure B.6.1.1/06–1: Biotransformation Pathways Based on Identified Metabolites of SYN545192



Note: Oxidation may occur before demethylation.
Some metabolites occur as glucuronide or sulphate conjugates

B.6.1.1 Toxicokinetic studies – single dose, oral route, in rats

Report:	IIA 5.1.1/01. Shaw J 2011a. SYN545192- An Investigation of the Tissue Distribution (QWBA) of Total Radioactivity in the Rat Following Oral Administration of Pyrazole or Phenyl Labelled [^{14}C]-SYN545192. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Charles River Report No. 30411. Issue date 12 August 2011. Unpublished (Syngenta File No.SYN545192_10125) EPA MRID No. 48604421

Guidelines: Metabolism – rat; OECD 417 (1984); EPA OPPTS 870.7485 (1998); 87/302/EEC (1987), B36, 94/79/EC (1994), JMAFF 12 Nohsan No 8147(2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

Four groups of 2 male and 2 female fasted Han Wistar rats were given a single oral dose of either [pyrazole-5-^{14}C]- or [phenyl-U-^{14}C]-SYN545192 at either 1 mg/kg or 40 mg/kg, to investigate the tissue distribution of total radioactivity. Dose formulations were suspensions in aqueous carboxymethylcellulose containing 0.1% Tween 80. Rats were humanely killed at two time points, *circa* Tmax and 3 days post dose, and residual radioactivity was measured in blood and selected tissues/organs by quantitative whole body autoradiography (QWBA). Expired air was measured over the first 24 hours after dosing with 1 mg/kg for both sexes and for both radiolabelled forms of SYN545192.

Following a single oral dose of 1 mg/kg, of either label of SYN545192 to fasted rats, the tissue distribution of radioactivity was extensive in both sexes. Peak tissue concentrations were attained at the first termination time (5 hours post dose in males and 1 hour post dose in females), when concentrations were generally higher in female tissues. The highest concentrations were present in the Harderian gland, liver and male bladder wall with lower concentrations in the kidney, brown fat and adrenal glands. By 72 hours post dose, all tissue concentrations had declined and the majority of tissue residues exceeded the circulating concentration in blood in females, with around half exceeding blood in males. Tissue concentrations were similar in both sexes.

Expired air accounted for 0.01% and 0.03% of the dose after 1 mg/kg doses of [pyrazole-5-^{14}C]- and [phenyl-U-^{14}C]-SYN545192, respectively. Therefore, expired air was considered a minor route of excretion with little to no differences between the moieties.

Following administration of SYN545192 at 40 mg/kg to fasted rats, for both radiolabelled forms, adverse clinical observations were observed mainly in the females and to a lesser extent in the males from around 1-6 hours post dose. Due to the severity of these clinical signs two of the female animals were prematurely killed at 5 hours post dose.

Following a single oral dose of 40 mg [pyrazole-5-^{14}C]- or [phenyl-U-^{14}C]-SYN545192/kg, radioactivity was, similar to the 1 mg/kg dose, widely distributed to the tissues in both sexes. Peak tissue concentrations were attained at the first termination time (5 hours post-dose in both

males and females). The highest concentrations were found in the Harderian gland and the liver with lower concentrations in the adrenal glands, brown fat, kidney and preputial gland. By 72 hours post dose, all tissue concentrations had declined and the majority of tissue residues exceeded the circulating concentration in blood in females, with most tissue concentrations below blood in males. Tissue concentrations were similar in both sexes.

Following both doses, the high concentrations of radioactivity in the gastrointestinal tract and its contents throughout the 72 hour time course were consistent with the established biliary elimination and faecal excretion of SYN545192 and its metabolites.

The high liver residues were consistent with the role of this organ in biotransformation and biliary elimination and the urinary bladder and kidney concentrations were consistent with the urinary excretion of SYN545192 related material.

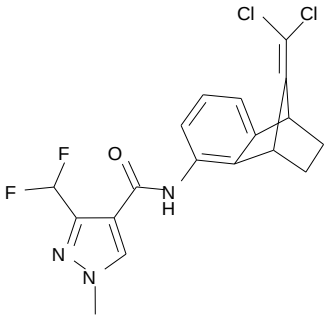
There were few clear differences in tissue distribution profiles between the [pyrazole-5-^{14}C]- or [phenyl-U-^{14}C]-SYN545192 and were few pronounced sex or dose differences. Total radioactivity was extensively distributed throughout the body with most tissue concentrations exceeding that in blood throughout the study. Tissue concentrations of radioactivity were highest at the first termination time (5 hours in males or 1 hour in females following the 1 mg/kg dose and 5 hours for both sexes following the 40 mg/kg dose) and had declined markedly by 72 hours post dose. In general, the highest tissue concentrations were present in the Harderian gland and liver with lower concentrations in the adrenal gland, brown fat and kidney.

Since, expired air accounted for only 0.01% and 0.03% of a 1 mg/kg dose of [pyrazole-5-^{14}C]- or [phenyl-U-^{14}C]-SYN545192, respectively, both radiolabelled positions are considered to be metabolically stable with respect to this route of excretion, with no volatile metabolites produced.

MATERIALS AND METHODS

Materials

Unlabelled Test Material: SYN545192
Physical state: White solid
Purity: 98.3%
Source: Syngenta Crop Protection Inc.
Lot/Batch number: TE-6341
Structure:

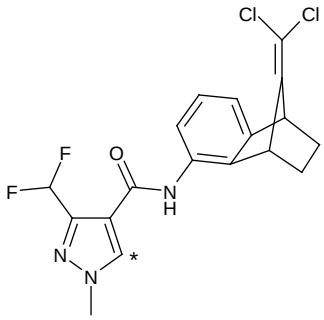


Unlabelled test item was used in the high dose preparations to dilute the specific activity of the radiolabelled test item

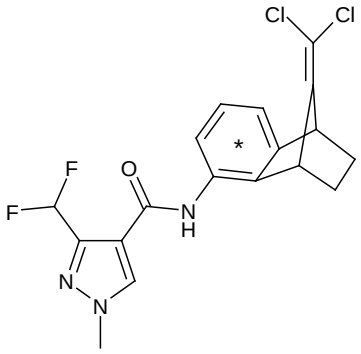
Radiolabelled Test Material: [Pyrazole-5-^{14}C]-SYN545192
Specific activity: Low Dose: 5.51 MBq/mg

High Dose: 0.141 MBq/mg

Radiochemical purity: 98.2%
Source: Selcia
Lot/Batch number: 5052PJS001-1
Structure:



* position of [^{14}C]-label

Radiolabelled Test Material:	[Phenyl-U-^{14}C]-SYN545192
Specific activity:	Low Dose: 5.51 MBq/mg High Dose: 0.128 MBq/mg
Radiochemical purity:	98.8%
Source:	Selcia
Lot/Batch number:	5051GAR006-4
Structure:	

* position of [^{14}C]-label

Vehicle: 1% carboxymethylcellulose (CMC) containing 0.1% (v/v) Tween 80.

Preparation of dosing solutions: Radiolabelled SYN545192 was homogenously suspended in 1% CMC containing 0.1% (v/v) Tween 80 for dosing.

Test Animals:

Species:	Rat
Strain:	Han Wistar
Age/weight at dosing:	8 or 10 weeks Low pyrazole dose: 281-298 g (males), 192-196 g (females) High pyrazole dose: 290-298 g (males), 170-198 g (females) Low phenyl dose: 222-224 g (males), 163-167 g (females) High phenyl dose: 221-224 g (males), 163-170 g (females)
Source:	Charles River (UK) Limited
Housing:	During the pre-study holding period, rats were multiply housed by sex in polycarbonate and stainless steel caging with bedding. Following dosing animals were housed in groups of 2 by sex in polycarbonate and stainless steel cages with raised wire mesh floors. Following dosing the animals intended for expired air collection were housed singly in all-glass metabolism cages. Due to adverse clinical signs, the 40 mg/kg dose animals were taken from expired air collection at around 2 hours post dose
Acclimatisation period:	5 days
Diet:	Rat and Mouse No.1 maintenance diet, Special Diet Services, Stepfield, Witham, Essex, UK. *Ad libitum* The animals were fasted approximately 12 hours before dosing. Following dosing the phase 1 and phase 3 animals (1 mg/kg), food was returned approximately 6 hours after dosing. Following dosing the phase 2 and phase 4 (40 mg/kg) animals, adverse clinical signs were witnessed from 1 hour post dose, and food was returned to the animals to try and help ease the clinical signs

Water:	Tap water *ad libitum*
Environmental conditions:	Temperature: 20-25°C Humidity: 36-63% (except for one occurrence of 108% on the day prior to dosing phase 1 and phase 3 animals) Air changes: At least 15 changes/hour Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

Experimental dates: Start: 06 February 2009 End: 22 April 2009

Group Arrangements: Animals were assigned to 4 groups as shown in the table below.

Table B.6.1.1/01–1: Dosing groups for pharmacokinetic studies for [^{14}C]-SYN545192

Test Group	Dose (mg/kg)	Number/sex	Remarks
Group 1 Pyrazole QWBA	1	2 males, 2 females	Whole body autoradiography terminated at 5 h (male), 1 h (female), and 72 h (both sexes). Also expired air collection at 0-24 h.
Group 2 Pyrazole QWBA	40	2 males, 2 females	Whole body autoradiography (sub-groups of 1 male and 1 female) terminated at 5 h[A] and 72 h
Group 3 Phenyl QWBA	1	2 males, 2 females	Whole body autoradiography terminated at 5 h (male), 1 h (female), and 72 h (both sexes)
Group 4 Phenyl QWBA	40	2 males, 2 females	Whole body autoradiography (sub-groups of 1 male and 1 female) terminated at 5 h[A] and 72 h. Also expired air collection at 0-24 h.

[A] = Female animals prematurely killed at 5 h due to adverse clinical signs, the target time had been 8 hours after dosing. Males were killed at 5 h to be consistent with the female kill time.

Dosing and sample collection:

A single oral dose of [^{14}C]-SYN545192 suspended in CMC containing Tween 80 was administered to each rat by gavage in a dose volume of 10 mL/kg. Animals in groups 1 and 3 received a dose corresponding to a nominal low dose of 1 mg/kg, and animals in groups 2 and 4 received a dose corresponding to a nominal high dose of 40 mg/kg. For both doses, the animals received a target radioactive dose of 5 MBq/kg.

Expired air was collected into monoethanolamine:ethanediol (3:7) traps for 1 male and 1 female from each group. Due to adverse clinical signs, the 40 mg/kg dose animals were taken from expired air collection at around 2 hours post dose and the expired air samples were not analysed.

Immediately following termination the right eye from each animal was removed, weighed and allowed to air dry at room temperature. Each carcass was then immediately frozen by immersion in a mixture of solid CO_2 in hexane for *ca* 30 min. Each frozen carcass was then embedded in a block of carboxymethylcellulose which was again frozen using the same freezing mixture. The frozen blocks were then stored in a freezer set to maintain -20°C prior to analysis.

A minimum of 3 sagittal sections at least 30µm thick were taken through each animal to ensure sampling of all major organs and tissues. The sections were freeze dried prior to exposure on storage phosphor screens. Duplicate aliquots of liver were removed from the remaining carcass following sectioning. Animals were sectioned such that the following tissues were present (where appropriate for each sex):

Adrenal	Ovary
Bladder	Pancreas
Blood	Pineal body
Bone Marrow	Pituitary gland
Brain	Preputial gland
Epididymis	Prostate
Eye	Rectum
Fat (brown)	Salivary gland
Fat (white)	Seminal vesicles
Harderian Gland	Skin
Heart	Small intestine
Muscle	Spinal cord
Kidney	Spleen
Lachrymal gland	Stomach
Large intestine	Testes
Liver	Thymus
Lung	Thyroid
Lymph node (neck)	Uterus
Muscle	Uveal Tract

Samples analysed for radioactivity by liquid scintillation counting was either directly or following sample oxidation.

Statistics: Not applicable.

RESULTS AND DISCUSSION

Pharmacokinetic Studies:

Preliminary experiment: Not applicable

Tissue distribution:

The concentrations of radioactivity in tissues and organs at various time points after single oral administration of pyrazole or phenyl labelled [^{14}C]-SYN545192 at a nominal dose of 1 mg/kg or 40 mg/kg are presented in the tables below.

In the pyrazole-labelled animals, the largest concentrations in males 5 hours following dose administration were in the bladder wall, small intestine wall, large intestine wall, liver, lachrymal gland, harderian gland and stomach wall. By 72 hours post-dosing, the largest concentrations were in the harderian gland, kidney cortex, kidney (whole) and liver, with the highest concentrations 200 – 10 x smaller than those at 5 hours. When compared to blood concentrations, 74% of the samples at the 5 hour mark were higher than blood concentrations in males and 45% of the samples were higher than blood at 72 hours. In females at 1 hour following dosing, the highest concentrations are in the liver, kidney cortex, brown fat, whole kidney, heart, harderian gland, adrenal cortex and whole adrenal. By 72 hours post-dosing, the highest concentrations were in the harderian gland, thyroid gland, heart, liver, brown fat and kidney cortex and the values were half those of the maximum values following 1 hour. In females, 97% of the samples were higher than blood at 1 hour and 78% were higher than blood at 72 hours.

In the phenyl-labelled animals, concentrations were within the same range with the exception of the amount of radioactivity found within the bladder wall of males 5 hours following dosing.

Table B.6.1.1/01–2: Distribution of radioactivity in tissues/organs 1, 5 and 72 hours after administration of [Pyrazole-^{14}C]-SYN545192 to male and female rats at a dose of 1 mg/kg

	µg equivalents of SYN545192/g tissue			
	Male		**Female**	
Tissue	**5 h (001M)**	**72 h (002M)**	**1 h (003F)**	**72 h (004F)**
Adrenal cortex	0.890	0.031	1.150	0.061
Adrenal medulla	0.764	0.034	0.675	0.046
Adrenal (whole)	0.871	0.032	1.114	0.058
Bladder wall	28.026	0.034	0.168	0.021
Blood	0.257	0.032	0.156	0.018
Bone Marrow	0.207	0.010	0.236	0.011

Brain	0.157	0.007	0.415	0.014
Brown fat	0.907	0.038	1.316	0.086
Epididymis	0.276	0.028	NA	NA
Eye	0.074	0.005	0.044	0.005
Harderian gland	1.268	0.149	1.258	0.678
Heart	0.892	0.053	1.263	0.095
Kidney cortex	0.877	0.123	1.347	0.082
Kidney medulla	0.755	0.075	0.845	0.040
Kidney (whole)	0.861	0.112	1.281	0.071
Lachrymal gland	1.280	0.041	0.825	0.050
Large intestine wall	2.452	0.020	0.346	0.068
Liver	1.311	0.106	1.767	0.095
Lung	0.335	0.079	0.165	0.026
Lymph node	0.321	0.027	0.375	0.023
Ovary	NA	NA	0.428	0.021
Pancreas	0.673	0.043	0.884	0.060
Pineal body	NP	0.027	0.480	NP
Pituitary gland	0.441	0.027	0.624	0.035
Preputial gland	0.432	0.040	0.418	0.074
Prostate	0.338	0.021	NA	NA
Rectum wall	0.193	0.018	0.485	0.059
Salivary gland	0.532	0.039	0.909	0.066
Seminal vesicles	0.202	0.012	NA	NA
Skeletal muscle	0.353	0.014	0.524	0.028
Skin	0.166	0.034	0.226	0.062
Small intestine wall	5.639	0.016	0.474	0.067

Spinal cord	0.127	0.011	0.440	0.016
Spleen	0.271	0.028	0.407	0.023
Stomach wall	1.210	0.023	0.464	0.017
Testes	0.275	0.010	NA	NA
Thymus	0.260	0.016	0.341	0.019
Thyroid gland	0.691	0.048	0.687	0.105
Uterus	NA	NA	0.229	0.015
Uveal tract	0.211	0.045	0.299	0.010
White fat	0.139	0.004	0.171	0.009
Limit of reliable measurement	0.001	0.002	0.001	0.001

NA = Not applicable, NP = Not present

Table B.6.1.1/01–3: Distribution of radioactivity in tissues/organs 1, 5 and 72 hours after administration of [Phenyl-^{14}C]-SYN545192 to male and female rats at a dose of 1 mg/kg

	µg equivalents of SYN545192/g tissue			
	Male		**Female**	
Tissue	**5 h (009M)**	**72 h (010M)**	**1 h (011F)**	**72 h (012F)**
Adrenal cortex	0.724	0.074	1.371	0.060
Adrenal medulla	0.621	0.068	0.965	0.039
Adrenal (whole)	0.719	0.070	1.283	0.057
Bladder wall	1.471	0.021	0.191	0.014
Blood	0.275	0.040	0.140	0.020
Bone Marrow	0.131	0.013	0.323	0.008
Brain	0.139	0.008	0.495	0.006
Brown fat	0.815	0.045	1.379	0.084
Epididymis	0.321	0.045	NA	NA
Eye	0.064	0.012	0.082	0.002
Harderian gland	1.548	0.322	1.425	0.820

Heart	0.741	0.055	1.488	0.060
Kidney cortex	0.655	0.151	1.191	0.093
Kidney medulla	0.358	0.075	0.691	0.048
Kidney (whole)	0.622	0.133	1.107	0.081
Lachrymal gland	0.329	0.051	0.871	0.042
Large intestine wall	7.605	0.050	1.124	0.108
Liver	0.950	0.095	1.875	0.089
Lung	0.327	0.065	0.074	0.021
Lymph node	0.319	0.024	0.403	0.019
Ovary	NA	NA	0.425	0.018
Pancreas	0.640	0.061	1.007	0.053
Pineal body	0.332	0.023	NP	NP
Pituitary gland	0.470	0.032	0.628	0.025
Preputial gland	0.407	0.025	0.628	0.046
Prostate	0.615	0.024	NA	NA
Rectum wall	0.247	0.018	0.555	0.020
Salivary gland	0.513	0.032	1.042	0.048
Seminal vesicles	0.418	0.010	NA	NA
Skeletal muscle	0.376	0.025	0.585	0.020
Skin	0.251	0.032	0.195	0.016
Small intestine wall	5.081	0.022	1.541	0.052
Spinal cord	0.170	0.008	0.348	0.011
Spleen	0.228	0.038	0.459	0.024
Stomach wall	0.361	0.041	0.486	0.034
Testes	0.123	0.009	NA	NA
Thymus	0.238	0.014	0.371	0.017

Thyroid gland	0.676	0.049	0.696	0.037
Uterus	NA	NA	0.240	0.015
Uveal tract	0.180	0.036	0.371	0.002
White fat	0.063	0.005	0.407	0.006
White tissue	0.180	0.009	NA	NA
Limit of reliable measurement	0.001	0.002	0.001	0.001

NA = Not applicable, NP = Not present

Five hours following dosing with 40 mg/kg bw per day [Pyrazole-^{14}C] SYN545192, the highest concentrations of radioactivity were found in the small intestine wall, large intestine wall, liver, harderian glands, adrenal cortex, whole adrenal, brown fat and stomach. By 72 hours, the highest concentrations were in the harderian glands, preputial gland, liver, kidney cortex, whole kidney and large intestine wall. There was a 3-fold decrease in highest tissues concentrations from 5 to 72 hours. Sixty-five percent of the tissues had concentrations higher than blood at 5 hours. By 72 hours, 37% of tissues had concentrations higher than blood. In females, the highest concentrations 1 hour post-dosing were in the small intestine wall, harderian gland, liver, adrenal cortex, preputial gland, whole adrenal, brown fat and large intestine wall. By 72 hours following dosing, the highest concentrations were in the pituitary gland, harderian gland and large intestine wall. The highest concentrations at 1 hour were 4 times lower than those at 72 hours. Ninety-two percent of the tissues had radioactivity concentrations higher than blood at 1 hour and, by 72 hours, that percentage was reduced to 61%.

Like the low dose, there were few major differences in radioactivity between the pyrazole and phenyl moieties. The exception was a much lower level of radioactivity found in the large intestine wall of the males 5 hours post-dosing.

Table B.6.1.1/01–4: Distribution of radioactivity in tissues/organs 5 and 72 hours after administration of [Pyrazole-^{14}C]-SYN545192 to male and female rats at a dose of 40 mg/kg

	µg equivalents of SYN545192/g tissue			
	Male		**Female**	
Tissue	**5 h (005M)**	**72 h (006M)**	**5 h (007F)**	**72 h (008F)**
Adrenal cortex	14.60	0.89	16.91	0.65
Adrenal medulla	8.16	0.96	10.06	0.24
Adrenal (whole)	14.12	0.92	15.51	0.61
Bladder wall	3.42	0.76	3.96	ND

Blood	3.92	0.91	1.89	0.24
Bone Marrow	3.43	0.26	2.33	0.20
Brain	2.71	0.26	6.64	ND
Brown fat	12.13	1.03	14.81	0.73
Epididymis	2.87	0.49	NA	NA
Eye	0.74	0.17	0.47	*0.00
Harderian gland	21.27	10.20	22.44	8.03
Heart	9.67	1.23	11.14	0.69
Kidney cortex	10.92	1.79	9.21	0.76
Kidney medulla	6.61	0.85	4.48	0.53
Kidney (whole)	10.37	1.69	8.24	0.73
Lachrymal gland	7.01	0.84	8.25	0.47
Large intestine wall	29.75	1.36	14.54	4.18
Liver	22.54	2.48	22.00	1.29
Lung	4.70	1.11	3.41	0.34
Lymph node	4.88	0.56	4.80	ND
Ovary	NA	NA	10.18	0.24
Pancreas	10.72	0.94	13.45	0.52
Pineal body	NP	NP	NP	NP
Pituitary gland	7.50	0.64	9.78	9.11
Preputial gland	5.24	3.15	16.17	1.64
Prostate	5.70	0.57	NA	NA
Rectum wall	5.49	0.93	6.94	0.35
Salivary gland	10.75	0.89	11.20	0.58
Seminal vesicles	1.88	0.27	NA	NA
Skeletal muscle	4.32	0.44	4.64	0.37

Skin	2.93	0.75	1.71	0.65
Small intestine wall	33.81	0.79	40.54	1.32
Spinal cord	2.90	0.37	7.46	ND
Spleen	3.87	0.49	5.11	0.32
Stomach wall	12.10	0.57	9.90	0.61
Testes	1.68	0.26	NA	NA
Thymus	4.14	0.41	4.51	ND
Thyroid gland	7.30	0.90	7.18	ND
Uterus	NA	NA	3.88	ND
Uveal tract	2.85	0.53	4.44	*0.04
White fat	3.84	0.11	4.04	*0.07
Limit of reliable measurement	0.04	0.06	0.02	0.07

*= below limit of reliable measurement, ND = Not discernible, NA = Not applicable, NP = Not present

Table B.6.1.1/01–5: Distribution of radioactivity in tissues/organs 5 and 72 hours after administration of [Phenyl-^{14}C]-SYN545192 to male and female rats at a dose of 40 mg/kg

	µg equivalents of SYN545192/g tissue			
	Male		**Female**	
Tissue	**5 h (013M)**	**72 h (014M)**	**5 h (015F)**	**72 h (016F)**
Adrenal cortex	12.44	1.43	30.07	1.42
Adrenal medulla	7.47	1.20	13.39	1.06
Adrenal (whole)	11.95	1.41	28.44	1.37
Bladder wall	4.70	0.53	10.91	0.44
Blood	2.52	1.37	3.17	0.67
Bone Marrow	1.83	0.25	7.98	0.23
Brain	1.78	0.24	7.82	0.17
Brown fat	10.71	1.47	21.10	1.88

Epididymis	2.34	0.80	NA	NA
Eye	0.38	0.11	0.84	0.43
Harderian gland	17.55	7.97	40.41	29.26
Heart	7.90	1.51	17.13	1.33
Kidney cortex	10.68	2.78	17.12	2.47
Kidney medulla	6.60	1.54	8.97	1.15
Kidney (whole)	10.42	2.60	12.21	2.25
Lachrymal gland	7.57	1.06	15.81	1.36
Large intestine wall	5.36	1.91	13.73	3.14
Liver	18.85	3.87	30.36	2.62
Lung	4.08	1.42	4.26	0.77
Lymph node	2.99	0.64	9.37	0.35
Ovary	NA	NA	12.25	0.58
Pancreas	8.95	1.05	19.59	1.21
Pineal body	NP	0.59	14.92	0.58
Pituitary gland	5.22	0.75	18.31	0.71
Preputial gland	8.84	0.48	19.98	1.83
Prostate	3.99	0.71	NA	NA
Rectum wall	3.13	0.77	11.55	0.35
Salivary gland	7.73	1.11	19.53	1.02
Seminal vesicles	3.36	0.21	NA	NA
Skeletal muscle (muscle)	3.60	0.44	10.35	0.53
Skin	1.83	0.96	5.73	1.22
Small intestine wall	20.14	1.19	36.88	3.68
Spinal cord	2.21	0.36	8.97	0.25
Spleen	3.62	0.66	9.42	0.75
Stomach wall	9.22	0.87	12.45	5.12

Testes	1.29	0.26	NA	NA
Thymus	2.64	0.39	8.49	0.43
Thyroid gland	6.03	1.17	16.73	0.90
Uterus	NA	NA	6.60	0.51
Uveal tract	1.54	0.35	7.91	0.38
White fat	1.60	0.17	7.89	0.28
White tissue	NA	NA	NA	NA
Limit of reliable measurement	0.06	0.04	0.09	0.08

NA = Not applicable, NP = Not present

Expired air:

Following a single oral dose of 1 mg pyrazole or phenyl labelled [^{14}C]-SYN545192/kg, 0.01% and 0.03% of the administered dose was recovered in expired air over the 0-24 hour collection period in both sexes, respectively.

CONCLUSION:

There were no clear differences in tissue distribution profiles between the [pyrazole-5-^{14}C]- or [phenyl-U-^{14}C]-SYN545192, nor were any pronounced sex or dose differences apparent. Total radioactivity was extensively distributed throughout the body with most tissue concentrations exceeding that in blood throughout the study. Tissue concentrations of radioactivity were highest at the first termination time (5 hours in males or 1 hour in females following the 1 mg/kg dose and 5 hours for both sexes following the 40 mg/kg dose) and had declined markedly by 72 hours post dose. In general, the highest tissue concentrations were present in the Harderian gland and liver with lower concentrations in the adrenal gland, brown fat and kidney.

Since, expired air accounted for only 0.01% and 0.03% of a 1 mg/kg dose of [pyrazole-5-^{14}C]- or [phenyl-U-^{14}C]-SYN545192, respectively, both positions of radiolabelling are considered to be metabolically stable with respect to this route of excretion, with no volatile metabolites produced.

Reviewer's Conclusions:

The reviewer agrees that there are few differences of note between the pyrazole and phenyl moieties in tissue distribution. The differences in bladder and intestinal radioactivity may indicate differences in faecal and urinary excretion in the first 5 hours in males, but otherwise the reviewer agrees with the investigators' decision to proceed with the pyrazole-labelled compound only.

Differences between the sexes are minor and may be a result of intraspecies variability rather than sex differences.

Report:	**IIA 5.1.1/05.** Shaw J & Montgomery E, 2011e. SYN545192 – The Biliary Elimination of Total Radioactivity in the Rat Following Single Oral Administration of [Pyrazole-^{14}C]-SYN545192. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Charles River Report Amendment 1 No. 30913. Issue date 12 August 2011. Unpublished (Syngenta File No. SYN545192_10122).EPA MRID No.48604422

Guidelines: Metabolism –Rat;

OECD 417 (1984);

EPA OPPTS 870.7485 (1998); 87/302/EEC B36 (1987), 94/79/EC (1994),

JMAFF 12 Nohsan No 8147 (2000)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

Two groups of 4 male and 4 female, Han Wistar, bile-duct cannulated (BDC) rats were given a single oral dose of 1 mg or 40 mg [pyrazole-5-^{14}C]-SYN545192/kg, to investigate the biliary elimination of radioactivity over two days. After this period, the rats were humanely killed and residual radioactivity was measured in blood, plasma, the gastrointestinal tract (and contents) and the remaining carcass.

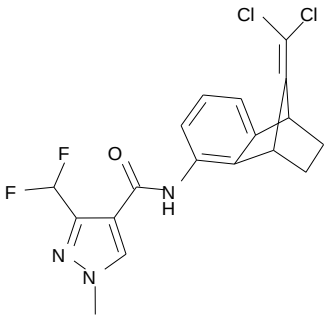
Following a single oral doses of 1 or 40 mg[pyrazole-5-^{14}C]-SYN545192/kg bw, the major route of excretion was the bile. In the low dose, 76.1 and 68.5% of the administered dose in males and females, respectively, was excreted via the bile. In the high dose, 47.3 and 56.5% of the administered dose was excreted via the bile in males and females, respectively. Faecal excretion comprised 16.9 and 15.8% of the administered 1 mg/kg bw dose in males and females and 32.7 and 31.9% of the administered 40 mg/kg bw dose in males and females. Biliary excretion peaked at 2 – 4 hours in low-dose animals and 8 – 24 hours in high-dose animals.

In conclusion, absorption was at least 79% of the administered dose following 1 mg [pyrazole-5-^{14}C]-SYN545192/kg bw and at least 60% of the administered dose following 40 mg [pyrazole-5-^{14}C]-SYN545192/kg bw.

MATERIALS AND METHODS

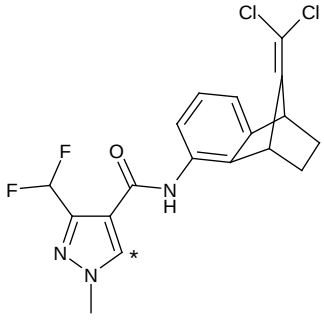
Materials:

Unlabelled Test Material:	SYN545192
Physical state:	Beige solid
Purity:	97%
Source:	Syngenta Crop Protection Inc.
Lot/Batch number:	SMU9BP005
Structure:	



Unlabelled test item was used in the high dose preparation to dilute the specific activity of the radiolabelled test item.

Radiolabelled Test Material:	[Pyrazole-5-^{14}C]-SYN545192
Specific activity:	Low Dose: 5.55 MBq/mg
	High Dose: 0.140 MBq/mg
Radiochemical purity:	>97%
Source:	Selcia
Lot/Batch number:	5107PJS001-1
Structure:	



* position of [^{14}C]-label

Vehicle: 1% carboxymethylcellulose (CMC) containing 0.1% (v/v) Tween 80.

Preparation of dosing solutions: Radiolabelled SYN545192 was homogenously suspended in 1% CMC containing 0.1% (v/v) Tween 80 for dosing.

Test Animals:

Species:	Rat
Strain:	Han Wistar
Age/weight at dosing:	8-12 weeks Low dose: 258-279 g (males), 201-209 g (females) High dose: 253-285 g (males), 223-241 g (females)
Source:	Charles River (UK) Limited (Group 1 Females, Group 2) Charles River (France) Limited (Group 1 Males)
Housing:	During the pre-study holding period, rats were singly housed in solid floored polycarbonate and stainless steel cages with bedding. Following external line attachment, animals were briefly housed singly in polypropylene and stainless steel cages Following dosing, animals were housed singly in all-glass metabolism cages designed for the separate qualitative collection of urine, bile and faeces
Acclimatisation period:	5 days
Diet:	Rat and Mouse No.1 maintenance diet, Special Diet Services, Stepfield, Witham, Essex, UK. *Ad libitum*
Water:	Tap water *ad libitum*
Environmental conditions:	Temperature: 21-22°C Humidity: 41 - 67% Air changes: At least 15 changes/hour Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

Experimental Dates: Start: 21 May 2009 End: 08 December 2009

Group Arrangements: Animals were assigned to 2 groups as shown in the table below.

Table B.6.1.1/05-1: Dosing groups for ADME studies for [^{14}C]-SYN545192

Test Group	Dose (mg/kg)	Number/sex	Remarks
Group 1 Bile duct cannulation (low dose)	1	4 males, 4 females	Excreta and bile collection over 2 days.
Group 2 Bile duct cannulation (high dose)	40	4 males, 4 females	Excreta and bile collection over 2 days.

Dosing and sample collection:

A single oral dose of [^{14}C]-SYN545192 suspended in CMC containing Tween 80 was administered to each rat by gavage in a dose volume of 10 mL/kg. Animals in group 1 received a dose corresponding to a nominal low dose of 1 mg/kg, and animals in group 2 received a dose corresponding to a nominal high dose of 40 mg/kg. For both doses, the animals received a target radioactive dose of 5 MBq/kg.

Urine and faeces were separately collected from individual rats. Urine, faeces and bile were frozen immediately upon collection (not including predose). At the end of each excreta collection period, cage wash samples were collected (water).

Animals were humanely killed by CO_2 narcosis. Each terminal blood sample was divided between two heparinised tubes, one of which was centrifuged to separate plasma. The gastrointestinal tract (and contents) and carcass were also retained separately.

All samples were analysed for radioactivity by liquid scintillation counting either directly or following sample oxidation.

Excretion studies:

Bile was collected at intervals of Pre-dose, 0-1, 1-2, 2-4, 4-8, 8-24, and 24-48 hours after dosing. Urine, faeces and cage wash were collected at daily intervals until the termination of the study.

To investigate pharmacokinetics, terminal blood samples were collected *via* the vena cava or by cardiac puncture at 48 hours after dosing.

Metabolite characterisation studies: Metabolite characterisation on selected plasma samples was undertaken in a separate study (Green, M and MacDonald, M 2011): SYN545192 - Investigation of the Nature and Identity of Radiolabelled Metabolites Present in Urine, Faeces, Bile and Plasma Collected from Rats Following Oral Administration of [^{14}C]-SYN545192. Charles River Report No. 31096. Unpublished. Syngenta File no. SYN545192_10131***, Annex B.6.1.1/06***.

Statistics: Not applicable.

RESULTS AND DISCUSSION

Absorption: The results of the urine, bile, cage wash and carcass samples were combined to estimate the amount of [^{14}C]-SYN545192 that was absorbed by the animals. Absorption was highest in the low dose males and females with 81.1 and 79.0%, respectively, of the administered radioactivity absorbed by the animals. In high-dose animals, 60.6 and 61.8% was absorbed in males and females, respectively.

There were no sex differences noted in overall absorption. While there were some apparent differences in the amount of radioactivity left in the carcass, these were not consistent in the low- and high-dose animals and more likely a result of intraspecies variability.

Table B.6.1.1/05-2: Absorption of radioactivity after administration of [^{14}C]-SYN545192

	Absorption after oral administration (Percent of radioactive dose)			
	Group 1 1 mg/kg		Group 2 40 mg/kg	
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=2)
Urine	3.6	4.0	8.6	3.8
Bile	76.1	68.5	47.3	56.5
Cage wash	0.5	0.4	1.5	0.2
Carcass	0.9	6.1	3.3	1.2
% Absorbed	81.1	79.0	60.6	61.8

Excretion:

The recovery of radioactivity in excreta and bile, following administration of a single oral dose of [^{14}C]-SYN545192 at doses of 1 or 40 mg/kg are presented in the table below.

As seen above, biliary excretion was the primary route of elimination in low-dose animals. It was essentially complete in low-dose males after 24 hours and reached its peak at 2 – 4 hours. In females, there was still some biliary excretion taking place after 24 hours, which is consistent with previous studies. Like the males, biliary excretion peaked at 2 – 4 hours after administration.

In the high-dose animals, 32 – 33% of the administered dose was excreted *via* the faeces without being absorbed. Biliary excretion peaked at 8 – 24 hours and there was significant excretion in the 24 – 28 hour time period.

Table B.6.1.1/05-3: Recovery of radioactivity in excreta and bile after administration of a single oral dose of [^{14}C]-SYN545192 to bile duct cannulated rats

		Group mean excretion data (percentage of radioactive dose recovered)			
		Group 1 1 mg/kg		Group 2 40 mg/kg	
		Male (n=4)	Female (n=4)	Male (n=4)	Female (n=2)
Urine	0-24 h	3.4	3.5	6.7	2.7
	24-48 h	0.2	0.5	1.9	1.1
	Subtotal	3.6	4.0	8.6	3.8
Faeces	0-24 h	16.3	14.3	18.6	17.8
	24-48 h	0.5	1.5	14.1	14.1
	Subtotal	16.9	15.8	32.7	31.9
Bile	0-1 h	7.8	<0.4	<0.1	<0.1
	1-2 h	18.0	12.6	0.9	1.5
	2-4 h	19.1	19.6	<2.1	2.6
	4-8 h	14.6	13.2	8.0	4.3
	8-24 h	14.7	16.2	26.0	31.0
	24-48 h	1.8	6.4	10.3	17.1
	Subtotal	76.1	68.5	47.3	56.5

Cage wash	0.5	0.4	1.5	0.2
GI tract + contents	<0.1	0.4	1.3	0.2
Carcass	0.9	6.1	3.3	1.2
Total Recovery	98.1	95.4	94.8	94.0

INVESTIGATORS' CONCLUSION:

After a single oral dose of [pyrazole-5-^{14}C]-SYN545192 at least 79% of the 1 mg/kg dose and at least 60% of the 40 mg/kg dose was absorbed. Irrespective of dose or sex, the radioactivity was fairly rapidly and extensively eliminated, predominantly *via* the bile. By two days after dosing carcass residues represented 6.1% of the dose or less.

REVIEWER'S CONCLUSIONS:

The reviewer agrees with the investigators' conclusions regarding the bililary excretion of [pyrazole-5-^{14}C]-SYN545192. Absorption was at least 79% of the administered dose in low-dose animals and at least 60% of the administered dose in high-dose animals. Biliary excretion was rapid with peaks in excretion between 2 and 4 hours in the low-dose animals and 8 – 24 hours in the high-dose animals. The bile is a major route of excretion in both doses and in both sexes.

Report:	IIA 5.1.1/03. Shaw J, 2011c. SYN545192- The Tissue Depletion of [Pyrazole-^{14}C]-SYN545192 in the Rat Following Single Oral Administration. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Charles River Report No. 30698. Issue date 11 August 2011. Unpublished (Syngenta File No.SYN545192_10124). EPA MRID No. 48604423

Guidelines: Metabolism – rat; OECD 417 (1984); EPA OPPTS 870.7485 (1998); 87/302/EEC (1987), B36, 94/79/EC (1994), JMAFF 12 Nohsan No 8147(2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

Two groups of 30 Han Wistar rats (15 per sex) were given a single oral dose of 1 mg or 40 mg [pyrazole-5-^{14}C]-SYN545192/kg in aqueous 1% carboxymethylcellulose containing 0.1% Tween 80 to investigate the tissue distribution of radioactivity. At intervals over a period of 6 days after dosing the rats were humanely killed in groups of 3 per sex and residual radioactivity was measured in selected tissues/organs and the remaining carcasses.

Following doses of 1 or 40 mg [pyrazole-5-^{14}C]-SYN545192/kg, radioactivity was well distributed throughout the tissues of males and females.

In males given 1 mg [pyrazole-5-^{14}C]-SYN545192/kg, peak concentrations occurred at 4 hours and elimination was consistent through 24 – 144 hours. Plasma concentrations were relatively low at 4 and 144 hours and just lower than liver and kidneys at 72 – 102 hours. In males given 40 mg [pyrazole-5-^{14}C]-SYN545192/kg, peak concentrations occurred at 24 hours and elimination was fastest between 24 and 48 hours. Plasma concentrations were proportionately highest at 48 hours and in the lower half of concentrations at 144 hours.

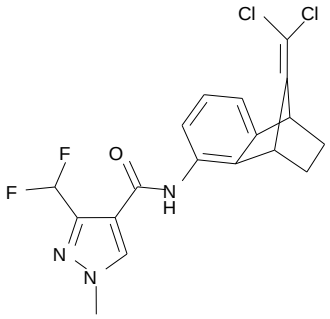
In females given 1 mg [pyrazole-5-^{14}C]-SYN545192/kg, peak radioactivity concentrations occurred at 4 hours and elimination was fastest between 24 and 72 hours. Plasma concentrations were proportionately low at all measured time points. In females given 40 mg [pyrazole-5-^{14}C]-SYN545192/kg, peak concentrations were at 24 hours and elimination was equally rapid at 24 – 48 hours and 48 – 72 hours. Plasma concentrations were proportionately highest at 72 hours, but low at all other time points.

The highest residues were in the organs related to metabolism and elimination in all doses and time points. After 144 hours, measureable amounts of radioactivity were found in almost all tissues.

In conclusion, absorption was rapid, distribution was extensive and elimination was rapid but incomplete. Elimination was fastest in between 4 and 24 hours in males and between 4 and 72 hours in females.

MATERIALS AND METHODS

Materials: SYN545192
Unlabelled Test Material:
Physical state: Beige solid
Purity: 97%
Source: Syngenta Crop Protection Inc.
Lot/Batch number: SMU9BP005
Structure:

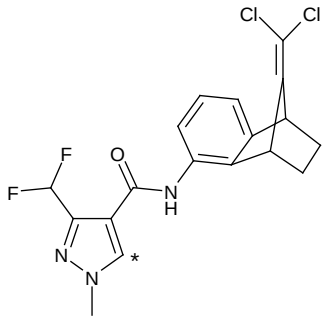


Unlabelled test item was used in the high dose preparation to dilute the specific activity of the radiolabelled test item

Radiolabelled Test Material: [Pyrazole-5-^{14}C]-SYN545192
Specific activity: Low Dose: 5.46 MBq/mg

High Dose: 0.118 MBq/mg

Radiochemical purity: >96%
Source: Selcia
Lot/Batch number: 5072PJS001-2
Structure:



* position of [^{14}C]-label

Vehicle: 1% carboxymethylcellulose (CMC) containing 0.1% (v/v) Tween 80.

Preparation of dosing solutions: Radiolabelled SYN545192 was homogenously suspended in 1% CMC containing 0.1% (v/v) Tween 80 for dosing.

Test Animals:

Species:	Rat
Strain:	Han Wistar
Age/weight at dosing:	7 weeks Low dose: 157-193 g (males), 118-145 g (females) High dose: 195-239 g (males), 172-201 g (females)
Source:	Charles River (UK) Limited
Housing:	During the pre-study holding period, rats were multiply housed by sex in polycarbonate and stainless steel caging with bedding. Following dosing animals were housed in groups of 3 in polycarbonate and stainless steel cages with raised wire mesh floors.
Acclimatisation period:	5 days
Diet:	Rat and Mouse No.1 maintenance diet, Special Diet Services, Stepfield, Witham, Essex, UK. *Ad libitum*
Water:	Tap water *ad libitum*
Environmental conditions:	Temperature: 21-22°C Humidity: 38-70% Air changes: At least 15 changes/hour Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

Experimental dates: Start: 21 May 2009 End: 30 November 2009

Group Arrangements: Animals were assigned to 2 groups as shown in the table below.

Table B.6.1.1/03-1: Dosing groups for pharmacokinetic studies for [^{14}C]-SYN545192

Test Group	Dose (mg/kg)	Number/sex	Remarks
Group 1 Tissue distribution	1	15 males, 15 females	Tissue collection (sub-groups of 3 males and 3 females) terminated at 4 h, 24 h, 72 h, 102 h, and 144 h
Group 2 Tissue distribution	40	15 males, 15 females	Tissue collection (sub-groups of 3 males and 3 females) terminated at 24 h, 48 h, 72 h, 102 h and 144 h

Dosing and sample collection:

A single oral dose of [^{14}C]-SYN545192 suspended in CMC containing Tween 80 was administered to each rat by gavage in a dose volume of 10 mL/kg. Animals in group 1 received a dose corresponding to a nominal low dose of 1 mg/kg, and animals in group 2 received a dose corresponding to a nominal high dose of 40 mg/kg. For both doses, the animals received a target radioactive dose of 5 MBq/kg.

Animals were humanely killed by CO_2 narcosis at various time points after dosing. A terminal blood sample was taken and divided between two heparinised tubes, one of which was centrifuged to separate plasma. The following tissues were taken for radioactivity analysis: adrenals, brain, heart, kidneys, liver, lungs, ovaries (females), pancreas, spleen, testes (males),

thymus, thyroid, uterus (females), gastrointestinal tract plus contents and residual carcasses together with representative samples of bone (tibia and fibula), renal fat and muscle.

All samples were analysed for radioactivity by liquid scintillation counting either directly or following sample oxidation.

Statistics: Not applicable.

RESULTS AND DISCUSSION

Pharmacokinetic Studies:

Preliminary experiment: No preliminary experiment was conducted.

Tissue distribution:

The concentrations of radioactivity in tissues and organs at various time points after single oral administration of [^{14}C]-SYN545192 at a nominal dose of 1 or 40 mg/kg are presented in the tables below.

In low-dose male animals, residues were at their peak concentrations at 4 hours. Elimination was consistent at 24, 72, 102 and 144 h in all tissues with the exception of the G.I. tract and contents where residues were slightly higher at 102 h than at 72 h. This is consistent with elimination. The highest concentrations were in the G.I. tract, contents, liver, adrenals and kidneys. Most tissues had residues higher than plasma levels at 4 hours and plasma concentrations were just slightly lower than those in the liver and kidney at 72 and 102 h. At 144 h, liver, kidney, G.I. tract, adrenal, heart, pancreatic, thyroid and lung residues were higher than plasma. There were still measurable amounts of radioactivity in all tissues at 144 h.

Table B.6.1.1/03-2: Distribution of radioactivity in tissues/organs 4, 24, 72, 102 and 144 hours after administration of [^{14}C]-SYN545192 to male rats at a dose of 1 mg/kg

	Group mean tissue residues (µg equiv/g or mL)				
Time after dosing	**4 h**	**24 h**	**72 h**	**102 h**	**144 h**
Adrenals	0.806	0.189	0.041	0.031	0.025
Bone Mineral	0.140	0.035	0.010	0.006	0.004
Brain	0.171	0.017	0.009	0.007	0.006
Fat-Renal	0.501	0.096	0.014	0.009	0.007
G.I. Tract	4.715	0.765	0.046	0.099	0.032
G.I Tract contents	7.327	2.262	0.080	0.224	0.069
Heart	0.699	0.123	0.041	0.030	0.023
Kidneys	0.806	0.178	0.074	0.058	0.039

Liver	1.168	0.233	0.073	0.055	0.043
Lungs	0.379	0.107	0.037	0.026	0.017
Muscle	0.362	0.062	0.017	0.014	0.010
Pancreas	0.576	0.113	0.036	0.027	0.021
Plasma	0.227	0.109	0.052	0.032	0.016
Residual carcass	0.284	0.067	0.026	0.018	0.014
Spleen	0.258	0.060	0.022	0.016	0.013
Testes	0.174	0.041	0.012	0.008	0.006
Thymus	0.235	0.053	0.015	0.009	0.007
Thyroid	0.611	0.125	0.049	0.026	0.021
Whole blood	0.174	0.079	0.036	0.024	0.015

In low-dose females, residues were also at peak concentrations at 4 hours. However, proportionately more radioactivity was removed from the tissues between 24 and 72 hours than between 4 and 24 hours. See Table B.6.1.1/03-3. The highest concentrations were in the G.I. tract and contents, liver, adrenals and kidneys like males; however, plasma levels were less than the majority of tissue samples at all time points. There were still measurable levels of radioactivity in all tissues with the exception of the bone and thyroid at 144 hours.

Table B.6.1.1/03-3: Distribution of radioactivity in tissues/organs 4, 24, 72, 102 and 144 hours after administration of [^{14}C]-SYN545192 to female rats at a dose of 1 mg/kg

	Group mean tissue residues (µg equiv/g or mL)				
Time after dosing	**4 h**	**24 h**	**72 h**	**102 h**	**144 h**
Adrenals	1.185	0.402 (↓66)	**0.071 (↓82)**	0.037 (↓48)	0.023 (↓38)
Bone Mineral	0.162	0.054 (↓67)	**0.013 (↓76)**	0.004 (↓69)	<0.003 (↓25)
Brain	0.290	**0.063 (↓78)**	0.015 (↓76)	0.006 (↓60)	0.003 (↓50)
Fat-Renal	0.824	0.225 (↓73)	**0.030 (↓87)**	0.019 (↓37)	0.011 (↓42)
G.I. Tract	4.813	1.008 (↓79)	**0.109 (↓89)**	0.043 (↓61)	0.042 (↓2)
G.I Tract contents	7.073	2.792 (↓61)	**0.133 (↓95)**	0.116 (↓13)	0.084 (↓28)
Heart	0.874	0.275 (↓69)	**0.054 (↓80)**	0.030 (↓44)	0.016 (↓47)
Kidneys	1.035	0.343 (↓67)	**0.070 (↓80)**	0.039 (↓44)	0.025 (↓36)

Liver	1.229	0.424 (↓66)	**0.078 (↓82)**	0.042 (↓46)	0.029 (↓31)
Lungs	0.383	0.149 (↓61)	**0.029 (↓81)**	0.015 (↓48)	0.010 (↓33)
Muscle	0.452	0.154 (↓66)	**0.028 (↓82)**	0.014 (↓50)	0.008 (↓43)
Ovaries	0.509	0.153 (↓70)	**0.029 (↓80)**	0.016 (↓45)	0.009 (↓44)
Pancreas	0.700	0.232 (↓67)	**0.050 (↓78)**	0.025 (↓50)	0.017 (↓32)
Plasma	0.167	0.077 (↓54)	**0.016 (↓79)**	0.009 (↓44)	0.005 (↓44)
Residual carcass	0.314	0.147 (↓53)	**0.032 (↓78)**	0.018 (↓44)	0.011 (↓39)
Spleen	0.319	0.116 (↓64)	**0.024 (↓79)**	0.013 (↓46)	0.009 (↓31)
Thymus	0.269	0.092 (↓66)	**0.020 (↓78)**	0.010 (↓50)	0.006 (↓40)
Thyroid	0.641	0.270 (↓58)	**0.042 (↓84)**	0.027 (↓36)	<0.012 (↓56)
Uterus	0.288	0.081 (↓72)	**0.020 (↓75)**	0.009 (↓55)	0.006 (↓33)
Whole blood	0.175	0.081 (↓54)	**0.017 (↓79)**	0.010 (↓41)	0.006 (↓40)

< = Below the limit of reliable measurement
( ) percent change from time period just previous

In the high-dose males, residues peaked at 24 hours and the rate of elimination was fastest between 24 and 48 hours. The highest concentrations of residues were in the G.I. tract and contents until 102 hours. The liver contained the next greatest concentrations of radioactivity and the highest concentrations at 102 and 144 hours. Plasma concentrations were never higher than liver and kidneys, were proportionately highest at 48 hours and were in the lower half of concentrations at 144 hours. Measurable concentrations of radioactivity were found in all tissues at 144 hours.

Table B.6.1.1/03-4: Distribution of radioactivity in tissues/organs 24, 48, 72, 102 and 144 hours after administration of [^{14}C]-SYN545192 to male rats at a dose of 40 mg/kg

	Group mean tissue residues (µg equiv/g or mL)				
Time after dosing	**24 h**	**48 h**	**72 h**	**102 h**	**144 h**
Adrenals	9.69	1.41	0.97	0.81	0.57
Bone Mineral	1.55	0.19	<0.12	<0.13	0.12
Brain	1.23	0.24	0.21	0.22	0.17
Fat-Renal	8.77	0.78	0.38	0.23	0.16
G.I. Tract	104.37	10.07	2.53	0.51	0.32
G.I Tract contents	157.40	23.73	3.91	0.74	0.35

Heart	5.33	1.26	1.02	0.85	0.67
Kidneys	8.18	2.19	1.67	1.51	1.16
Liver	14.13	2.92	1.96	1.63	1.39
Lungs	4.87	1.12	0.82	0.58	0.43
Muscle	2.99	0.54	0.41	0.38	0.27
Pancreas	5.84	1.22	0.86	0.89	0.66
Plasma	4.40	1.46	1.15	0.60	0.33
Residual carcass	4.16	0.97	0.60	0.51	0.39
Spleen	3.11	0.69	0.50	0.50	0.37
Testes	2.06	0.38	0.26	0.20	0.13
Thymus	2.65	0.49	0.34	0.29	0.22
Thyroid	7.78	1.27	0.93	0.51	0.80
Whole blood	3.13	1.06	0.87	0.56	0.38

In high-dose females, the rate of excretion was similar between the 24 – 48 and 48 – 72 hour time points. The highest proportions of radioactivity were always in the G.I. tract contents, followed by the G.I. tract itself, with the exception of the 104 hour mark where liver and kidney concentrations were slightly higher than the G.I. tract. At 72 hours, plasma concentrations were proportionately at their highest, but, on average, more tissues had higher concentrations than plasma. At the end of 144 hours, the only tissues without quantifiable levels of radioactivity were bone mineral and thyroid.

Table B.6.1.1/03-5: Distribution of radioactivity in tissues/organs 24, 48, 72, 102 and 144 hours after administration of [^{14}C]-SYN545192 to female rats at a dose of 1 mg/kg

	Group mean tissue residues (µg equiv/g or mL)				
Time after dosing	**24 h**	**48 h**	**72 h**	**102 h**	**144 h**
Adrenals	20.21	6.19	1.69	1.00	1.12
Bone Mineral	2.11	0.41	0.15	<0.09	<0.17
Brain	4.52	0.85	0.24	0.13	0.14
Fat-Renal	19.64	4.71	1.00	0.66	0.55
G.I. Tract	147.26	50.06	6.48	1.17	4.41
G.I Tract contents	408.19	107.84	11.96	2.98	9.59

Heart	9.69	3.36	1.20	0.85	0.82
Kidneys	13.26	4.67	1.90	1.29	1.25
Liver	25.22	7.80	2.35	1.43	1.65
Lungs	8.43	2.79	0.91	0.59	0.57
Muscle	5.00	1.68	0.46	0.33	0.36
Ovaries	8.53	2.81	0.81	0.43	0.50
Pancreas	12.89	4.04	1.27	0.90	0.86
Plasma	3.91	1.75	0.88	0.54	0.29
Residual carcass	6.53	2.96	0.86	0.61	0.64
Spleen	6.41	2.06	0.65	0.44	0.45
Thymus	5.24	1.84	0.50	0.27	0.37
Thyroid	10.96	3.69	1.08	<0.65	<0.64
Uterus	6.93	1.84	0.60	0.33	0.29
Whole blood	4.02	1.72	0.73	0.47	0.35

< = Below the limit of reliable measurement

Tissue elimination:

Half-lives in tissue were difficult to determine in 40 mg/kg bw/d females, as the regression line was a poor fit. The values expressed in Table B.6.1.1/03-6 were a best estimate.

Plasma half-lives were consistent irrespective of dose or sex and were in the 42.72-44.49 hour range. Low-dose females had the lowest half-lives, ranging from 33.57 hours in the brain to 61 hours in the heart. In low-dose males and high-dose males and females, the lowest half-lives were in the plasma. The highest were in the muscle in low-dose males, the brain in high-dose males and the thyroid in high-dose females. Half-lives in high-dose males and females were higher than those in low-dose animals, and half-lives in high-dose females were the highest of all.

Half-lives in the target organs (liver, kidney and thyroid) were as follows: 97.17 h, 75.01 h and 60.66 h, respectively, in low-dose males; 50.83 h, 49.56 h and 46.86 h, respectively, in low-dose females, 148.02 h, 136.04 h and 129.6 h, respectively in high-dose males; and 158.98 h, 126.86 h and 315.92 h in high-dose females. The brain had the most varied half-lives, ranging from 33.57 h in low-dose females to 209.88 h in high-dose males.

Table B.6.1.1/03-6: Elimination of radioactivity from rat tissues/organs after single administration of [^{14}C]-SYN545192 to rats at a dose of 1 mg/kg or 40 mg/kg

	Values are expressed as T½el(h). Each value is a mean of 3 rats			
	1 mg/kg		40 mg/kg	
Tissue	**Male**	**Female**	**Male**	**Female**
Adrenals	98.20	44.94	92.48	132.91*
Bone Mineral	49.95	39.41	164.44	136.06*
Brain	77.31	33.57	209.88	102.41*
Fat-Renal	71.06	48.49	58.72	85.42
Heart	88.69	61.00	108.49	137.53
Kidneys	75.01	49.56	136.04	126.86
Liver	97.17	50.83	148.02	158.98*
Lungs	62.76	47.35	70.88	114.43
Muscle	101.04	40.09	102.84	231.24*
Ovaries	NA	41.75	NA	112.82*
Pancreas	95.45	49.40	123.98	136.40
Plasma	43.61	42.72	43.06	44.49
Spleen	97.31	52.23	118.22	144.20*
Testes	60.19	NA	63.69	NA
Thymus	73.50	41.78	110.57	201.08*
Thyroid	60.66	46.86	129.60*	315.92*
Uterus	NA	43.04	NA	69.88
Whole Blood	54.76	50.29	62.40	70.61

NA = Not applicable * = Estimation of the terminal elimination phase slope deemed unreliable by the Pharmacokineticist, with the regression line (Rsq) also being of poor fit (<0.70)

INVESTIGATORS' CONCLUSION:

Irrespective of dose or sex, the tissue distribution of radioactivity was extensive following a single oral dose of 1 or 40 mg [^{14}C]-SYN545192/kg to rats. Tissue concentrations of radioactivity were highest at the first sampling time (4 hours post 1 mg/kg and 24 hours post 40 mg/kg) and progressively declined thereafter with elimination half lives of between 1.4 days and 8.7 days. By 144 hours post dose most tissue concentrations were still reliably detectable but were low, with total tissue and carcass residues accounting for less than 3.8% of the dose. The high concentrations of radioactivity in the gastrointestinal tract and its contents were consistent with the established biliary elimination and faecal excretion of SYN545192 and its metabolites.

REVIEWER'S CONCLUSION:

Tissue distribution was extensive regardless of dose or sex. While tissue concentrations peaked in the first sampling period in males and females (4 hours in low-dose animals and 24 hours in high-dose animals), elimination was more rapid through the first to second sampling period in males (4 – 24 h in low-dose animals and 24 – 48 h in high-dose animals) and stretched to the third sampling period in females (24 – 48 h in low-dose animals and 48 – 72 h in high-dose animals.) At the end of the sampling period, small amounts of radioactivity were still found distributed throughout the tissue samples.

Half-lives were shortest in low-dose females and highest in high-dose females. Plasma had the most rapid half-lives in low-dose males and high-dose males and females. While the brain had the most rapid half-lives in low-dose females, it also had the most variable half-lives. The longest half-lives were in the muscle, heart, brain and thyroid of the low-dose males and females and high-dose males and females, respectively.

B.6.1.3

Report:	IIA 5.1.3. Shaw J, 2011f. SYN545192- The Tissue Distribution and Elimination of [Pyrazole-^{14}C]-SYN545192 in the Rat Following Repeated Daily Oral Administration. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Charles River Report No. 31050. Issue date 09 August 2011. Unpublished (Syngenta File No.SYN545192_10120).EPA MRID No. 48604424

Guidelines: Metabolism – rat; OECD 417 (1984); EPA OPPTS 870.7485 (1998); 87/302/EEC (1987), B36, 94/79/EC (1994), JMAFF 12 Nohsan No 8147(2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

Thirty six male Han Wistar rats were given up to 14 consecutive daily oral doses of 1 mg [pyrazole-^{14}C]-SYN545192/kg. At predetermined intervals during dosing and following the cessation of dosing, groups of rats were humanely killed for the removal of selected tissues/organs to determine the extent of accumulation of radioactivity in tissues and the remaining carcasses and its subsequent elimination.

Additionally, the excretion of radioactivity in urine and faeces was monitored in one group of rats for a period of 24 hours following the first and fourteenth doses. The nature of radioactivity present in these key samples of urine and faeces was also determined at Charles River under a separate study (Charles River Study No. 214808) and was reported separately.

Following the repeated administration of 1 mg [pyrazole-^{14}C]-SYN545192/kg, the highest concentrations in the tissues occurred 24 hours following the last of the doses. After 3 days of dosing, the highest concentrations were in the kidneys, liver, thyroid and plasma. This was consistent through the 14 days of dosing, though adrenal levels were equally high by Day 14.

Following a 63 day recovery period, there were measurable amounts of radioactivity in all tissues except for plasma, whole blood, bone mineral and G.I. tract contents. The highest remaining concentrations were in the thyroid, adrenals and spleen.

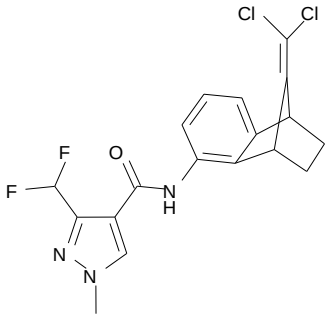
Tissue half-lives were shortest in the plasma at 2.49 days and longest in the testes at 69 days. Due to the low levels of radioactivity, some of the regression (Rsq) values were lower than ideal, but the best fit was in the renal fat with a half-life of 36.19 days.

After one day of dosing, animals excreted 78% of the administered dose. By day 14, animals excreted 118% of the administered dose. The majority of the increased excretion was in the faeces (from 72 – 110.5% of the administered dose), perhaps indicating an increase in excretion as well as a lack of absorption.

MATERIALS AND METHODS

Materials:

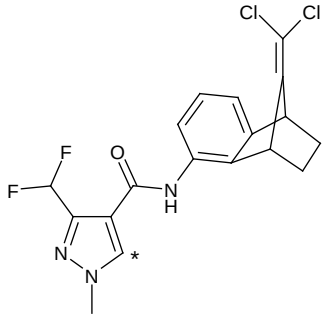
Unlabelled Test Material: SYN545192
Physical state: Beige solid
Purity: 97%
Source: Syngenta Crop Protection Inc.
Lot/Batch number: SMU9BP005
Structure:



Unlabelled test item was used to dilute the specific activity of the radiolabelled test item

Radiolabelled Test Material: [Pyrazole-5-^{14}C]-SYN545192
Specific activity: 1.909 MBq/mg

Radiochemical purity: >98%
Source: Selcia
Lot/Batch number: 5120PJS001-1
Structure:



* position of [^{14}C]-label

Vehicle: 1% carboxymethylcellulose (CMC) containing 0.1% (v/v) Tween 80.

Preparation of dosing solutions: Radiolabelled SYN545192 was homogenously suspended in 1% CMC containing 0.1% (v/v) Tween 80 for dosing.

Test Animals:

Species:	Rat
Strain:	Han Wistar
Age/weight at dosing:	7 weeks at first dose Weight range at time of first dose: 181 – 215 g
Source:	Harlan (UK) Limited
Housing:	During the pre-trial holding period, rats were multiply housed in polycarbonate and stainless steel caging with bedding. Following dosing animals were housed in groups of 3 in polycarbonate and stainless steel cages with raised wire mesh floors. Animals used to investigate excretion kinetics were singly housed in glass metabolism cages, before being returned to the original stainless steel housing.
Acclimatisation period:	5 days
Diet:	Rat and Mouse No.1 maintenance diet, Special Diet Services, Stepfield, Witham, Essex, UK. *Ad libitum*
Water:	Tap water *ad libitum*
Environmental conditions:	Temperature: 20-22°C Humidity: 31-68% Air changes: At least 15 changes/hour Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

Experimental dates: Start: 02 December 2009 End: 08 April 2010

Group Arrangements: Animals were assigned to 2 groups as shown in the table below.

Table B.6.1.3-1: Dosing groups for pharmacokinetic studies for [^{14}C]-SYN545192

Test Group	Dose (mg/kg)	Number/sex	Remarks
Tissue distribution	1	33 males	Tissue collection (sub-groups of 3 males terminated 24 hours after administration on Day 3, Day 7, Day 10 and Day 14, and 3, 7, 10, 14, 18, 21, and 28 days after administration on Day 14)
Excretion and tissue distribution		3 males	Excreta collection (0-24 hours post-dose of Day 1 and Day 14) and tissue collection 63 days after administration on Day 14

Dosing and sample collection:

A single daily oral dose of [^{14}C]-SYN545192 suspended in CMC containing Tween 80 was administered to each rat by gavage in a dose volume of 10 mL/kg for up to 14 consecutive days. Animals received a dose corresponding to a nominal dose of 1 mg/kg and a target radioactive dose of 2 MBq/kg.

Animals were humanely killed by CO_2 narcosis at various time points after the dosing initiation. A terminal blood sample was taken and divided between two heparinised tubes, one of which was centrifuged to separate plasma. The following tissues were taken for radioactivity analysis: adrenals, brain, heart, kidneys, liver, lungs, pancreas, spleen, testes, thymus, thyroid, gastrointestinal tract plus contents and residual carcasses together with representative samples of bone (tibia and fibula), renal fat and muscle.

Urine and faeces were separately collected from 3 animals subject to excreta collection over the 0-24 hours interval after Day 1 and Day 14. Urine and faeces were frozen immediately upon collection.

All samples were analysed for radioactivity by liquid scintillation counting either directly or following sample oxidation.

Metabolite characterisation studies:

Metabolite characterisation on selected plasma samples was undertaken in a separate study (Green, M and MacDonald, M 2011) SYN545192 - Investigation of the Nature and Identity of Radiolabelled Metabolites Present in Urine, Faeces, Bile and Plasma Collected from Rats Following Oral Administration of [^{14}C]-SYN545192. Charles River Report No. 31096. Unpublished. Syngenta File no. SYN545192_10131; ***Annex B.6.1.1/06***.

Statistics: Not applicable.

RESULTS AND DISCUSSION

Pharmacokinetic Studies:

Preliminary experiment: Not applicable

Tissue distribution:

The concentrations of radioactivity in tissues and organs at various timepoints after repeated oral administration of [^{14}C]-SYN545192 for up to 14 days at a nominal dose of 1 mg/kg are presented in the tables below.

Table B.6.1.3-2: Distribution of radioactivity in tissues/organs 24 hours after Day 3, 7, 10 and 14 of a repeated administration of [^{14}C]-SYN545192 to male rats at a dose of 1 mg/kg

	Group mean tissue residues (µg equiv/g or mL)			
Dose schedule	**Day 3**	**Day 7**	**Day 10**	**Day 14**
Adrenals	0.241	0.321	0.355	0.514
Bone Mineral	0.050	0.043	0.062	0.073
Brain	0.032	0.056	0.063	0.090

Fat-Renal	0.115	0.179	0.221	0.321
G.I. Tract	0.988	1.202	1.265	1.336
G.I Tract contents	2.405	2.921	2.620	3.225
Heart	0.189	0.252	0.302	0.420
Kidneys	0.301	0.499	0.515	0.702
Liver	0.393	0.584	0.560	0.776
Lungs	0.174	0.294	0.260	0.355
Muscle	0.080	0.131	0.125	0.169
Pancreas	0.178	0.305	0.273	0.369
Plasma	0.283	0.457	0.337	0.535
Residual carcass	0.125	0.193	0.181	0.270
Spleen	0.100	0.191	0.157	0.230
Testes	0.058	0.090	0.087	0.115
Thymus	0.073	0.117	0.120	0.164
Thyroid	0.283	0.471	0.290	0.517
Whole blood	0.190	0.307	0.252	0.379

Table B.6.1.3-3: Distribution of radioactivity in tissues/organs up to 63 days after Day 14 of repeated administration of [^{14}C]-SYN545192 to male rats at a dose of 1 mg/kg

		Group mean tissue residues (µg equiv/g or mL)						
Time after Day 14 dosing	**Day 3**	**Day 7**	**Day 10**	**Day 14**	**Day 18**	**Day 21**	**Day 28**	**Day 63**
Adrenals	0.253	0.134	0.152	0.139	0.132	0.069	0.125	0.070
Bone Mineral	0.054	0.021	0.017	0.018	0.014	0.011	0.012	<0.004
Brain	0.067	0.046	0.042	0.047	0.032	0.026	0.030	0.017
Fat-Renal	0.092	0.055	0.042	0.044	0.033	0.029	0.025	0.013
G.I. Tract	0.267	0.072	0.039	0.036	0.028	0.020	0.021	0.010
G.I Tract contents	0.462	0.083	0.023	0.020	0.009	0.008	0.007	<0.001
Heart	0.230	0.129	0.118	0.111	0.078	0.056	0.055	0.015

Kidneys	0.454	0.249	0.245	0.196	0.130	0.091	0.091	0.027
Liver	0.416	0.279	0.217	0.180	0.136	0.108	0.145	0.023
Lungs	0.192	0.111	0.083	0.085	0.060	0.045	0.049	0.017
Muscle	0.088	0.052	0.051	0.047	0.033	0.025	0.026	0.009
Pancreas	0.204	0.110	0.100	0.090	0.053	0.039	0.041	0.010
Plasma	0.228	0.072	0.026	0.011	0.003	<0.002	<0.001	<0.001
Residual carcass	0.136	0.089	0.069	0.073	0.050	0.044	0.048	0.018
Spleen	0.148	0.105	0.101	0.107	0.081	0.068	0.104	0.043
Testes	0.058	0.027	0.025	0.026	0.021	0.015	0.019	0.012
Thymus	0.080	0.045	0.045	0.036	0.027	0.020	0.021	0.013
Thyroid	0.258	0.125	0.217	0.120	0.179	0.092	0.124	0.092
Whole blood	0.201	0.107	0.079	0.071	0.050	0.041	0.041	<0.001

As summarised in Table B.6.1.3-2, the highest concentrations were found 24 hours following the last repeat dose (14^{th}). The brain had the highest increases over the day 3 residues at the end of the dosing period, followed by renal fat. After cessation of dosing, there was a steady decrease in residues in all tissues, though almost all tissues had measurable amounts of radioactivity 63 days after the cessation of dosing. The highest concentrations during dosing were in the liver and kidneys. Three days following the cessation of dosing, kidney, liver, thyroid, adrenal and heart tissues had the highest concentrations. Roughly mid-way through the recovery period, liver, adrenal, thyroid, spleen, kidney and heart tissues had the highest concentrations. By the end of the 63 day recovery period, thyroid, adrenal, spleen, kidney and liver tissues were the highest and all tissues with measurable values were above those in plasma and whole blood.

As summarised above, following a repeated oral administration of [^{14}C]-SYN545192 at target dose of 1 mg/kg/day, highest concentrations of radioactivity were observed at 24 h following the fourteenth and final dose, and most mean tissue concentrations appeared to be approaching steady state concentrations by the end of the 14 day dosing period. Following the cessation of dosing, all tissue concentrations steadily declined.

Tissue elimination:

The half-lives of elimination from tissues and organs following oral multiple administration of [^{14}C]-SYN545192 at a dose of 1 mg/kg are presented in the table below.

Table B.6.1.3-4: Elimination of radioactivity from rat tissues/organs after multiple administration of [^{14}C]-SYN545192 to rats at a dose of 1 mg/kg

	Half-life values for the elimination of radioactivity from tissues	
Tissues/organs	**T½ (days)**	**Rsq**
Adrenals	NC	0.28
Bone Mineral	22.05	0.97
Brain	49.59	0.92
Fat-Renal	36.19	1.00
Heart	19.65	0.98
Kidneys	21.42	0.97
Liver	17.30	0.91
Lungs	26.61	0.95
Muscle	24.49	0.97
Pancreas	18.65	0.97
Plasma	2.49	0.99
Spleen	NC	0.63
Testes	69.06	0.66
Thymus	61.77	0.95
Thyroid	NC	0.42
Whole Blood	NC	0.53

NC = Not calculated
Rsq the square of the correlation co-efficient for the terminal phase regression line.

The half-lives of radioactivity in the tissue varied greatly, from 2.49 days for the plasma to 69.09 days for the testes. The low concentrations in tissues at through the recovery period resulted in low Rsq values. The most reliable $t_{1/2}$ values renal fat, plasma and heart, followed by the bone mineral, kidneys, muscle and pancreas.

Excretion:

The recovery of radioactivity in excreta following multiple oral administration of [^{14}C]-SYN545192 at nominal doses of 1 mg/kg is presented in the tables below.

Table B.6.1.3-5: Recovery of radioactivity in excreta after multiple administration of [^{14}C]-SYN545192 to rats a dose of 1 mg/kg

		Group mean excretion data (percentage of radioactive dose recovered)	
		Day 1	**Day 14**
		Male (n=3)	**Male (n=3)**
Urine	0-24 h	5.1	5.9
Faeces	0-24 h	72.0	110.5
Cage wash		0.9	1.4
Total Excreted		78.0	117.8

The excreta were analysed for radioactivity on Day 1 and Day 14 of the repeat dose study. On Day 1, the animals excreted 78% of the administered radioactivity, with 72% in the faeces, 5.1% in the urine and 0.9% in the cage wash. On Day 14, the animals excreted 5.9% of the administered radioactivity in the urine, 110.5% in the faeces and 1.4% was found in the cage wash. This comprised 117.8% of the administered dose and was considered evidence that the animals were excreting residual radioactivity from previous doses.

Investigator's Conclusion:

Following repeated daily oral administration of 1 mg [^{14}C]-SYN545192/kg to male rats, tissue distribution of radioactivity was extensive and most tissue concentrations appeared to be approaching steady state concentrations after 14 doses. Tissue concentrations of radioactivity were highest in the liver followed by the kidney during dosing with all tissue concentrations declining following the cessation of dosing. By the final sampling time, concentrations were measurable in most tissues, but were approaching the limit of reliable measurement. The total tissue and carcass residues at the final sampling time accounted for less than 0.2% of the total radiolabelled dose administered. The terminal half-lives for tissue depletion were variable reflecting the low concentrations at or around the limit of reliable measurement for several tissues and ranged from 2.5 days for plasma to 69.1 days for the testes.

Reviewer's Conclusions:

The reviewer agrees with the investigator that the tissue distribution was extensive; however, the reviewer does not see evidence of approaching steady state concentrations.

The liver and kidney had the highest concentrations of radioactivity through the 14 days of dosing. Following the recovery period the thyroid, adrenal, spleen, kidney and liver tissues were the highest. Almost all tissues had measurable levels of radioactivity following 63 days of recovery and all tissues had levels above those in plasma and whole blood.

Tissue half-lives ranged from 2.49 days in the plasma to 69 days in the testes.

Analysis of the excreta indicated that the animals were capable of excreting more than the administered dose (total excreted = 117.8%) after 14 days of dosing.

[Flag to reviewers: At the section head presentation, we really couldn't come to a decision as to whether benzovindiflupyr bioaccumulated. It's going to JMPR this year between Sept 17 – 28 and the preliminary documentation also doesn't have a decision whether it bioaccumulates.

One peer reviewer thinks that the day 10 and day 14 values are similar enough that subsequent doses wouldn't increase the tissue concentrations.

Thoughts?]

Report:	IIA 5.1.1/02. Shaw J, 2011b. SYN545192 – The Excretion and Tissue Distribution of [^{14}C]-SYN545192 in the Rat Following Single Oral Administration. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Charles River Report Amendment 1 No. 30575. Issue date 12 August 2011. Unpublished (Syngenta File No. SYN545192_10123). EPA MRID No. 48604425

Guidelines: Metabolism – rat; OECD 417 (1984); EPA OPPTS 870.7485 (1998); 87/302/EEC (1987), B36, 94/79/EC (1994), JMAFF 12 Nohsan No 8147(2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

Two groups of 4 male and 4 female Han Wistar rats were given a single oral dose of 1 mg or 40 mg [pyrazole-5-^{14}C]-SYN545192/kg in 1% aqueous carboxymethylcellulose (CMC) containing 0.1% Tween 80 to investigate the excretion of radioactivity over seven days. After this period, the rats were humanely killed and residual radioactivity was measured in blood, selected tissues and the remaining carcasses.

The major route of elimination regardless of sex or dose was *via* the faeces, comprising 84.1 – 92.7% of the administered dose. Faecal elimination was rapid, occurring predominantly in the first 48 hours in low- and high-dose males and low-dose females. Faecal elimination was predominantly in the first 72 hours in high-dose females. Urinary excretion accounted for 6.3 – 12.2% of the administered dose and occurred predominantly in the first 24 hours.

Of the 0.9 – 1.9% of administered dose recovered in the tissues, carcass and G.I. tract with contents, the liver was found to contain the highest percentage of administered dose in low- and high-dose males and high-dose females. Low-dose females had higher percentages in the G.I. contents, followed by the liver. The largest percentages of administered dose, regardless of sex or dose, were found in the organs of excretion and metabolism, the G.I. tract, G.I. contents, liver and kidneys, which is well correlated with the rapid and extensive elimination of the test substance. Small amounts of [pyrazole-5-^{14}C]-SYN545192 were distributed extensively through the remaining tissues.

MATERIALS AND METHODS

Materials:

Unlabelled Test Material: SYN545192

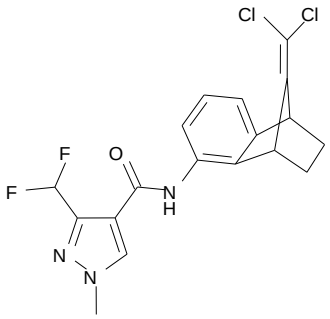
Physical state: Beige solid

Purity: 97%

Source: Syngenta Crop Protection Inc.

Lot/Batch number: SMU9BP005

Structure:



Unlabelled test item was used in the high dose preparation to dilute the specific activity of the radiolabelled test item

Radiolabelled Test Material: [Pyrazole-5-14C]-SYN545192

Specific activity: Low Dose: 5.46 MBq/mg

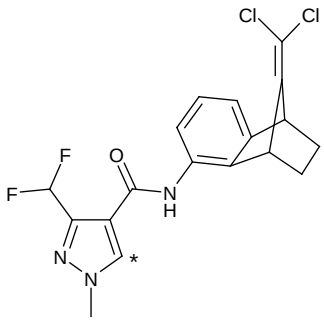
High Dose: 0.125 MBq/mg

Radiochemical purity: >98%

Source: Selcia

Lot/Batch number: 5072PJS001-2

Structure:



* position of [14C]-label

Vehicle: 1% carboxymethylcellulose (CMC) containing 0.1% (v/v) Tween 80.

Preparation of dosing solutions: Radiolabelled SYN545192 was homogenously suspended in 1% CMC containing 0.1% (v/v) Tween 80 for dosing.

Test Animals:

Species:	Rat
Strain:	Han Wistar
Age/weight at dosing:	7 weeks (low dose), 7-8weeks (high dose)
	Low dose: 223-253 g (males), 170-183 g (females)
	High dose: 209-246 g (males), 146-164 g (females)
Source:	Charles River (UK) Limited
Housing:	During the pre-study holding period, rats were multiply housed by sex in polycarbonate and stainless steel cages with bedding. Following dosing animals were housed singly in all-glass metabolism cages designed for the separate quantitative collection of urine and faeces
Acclimatisation period:	7 days
Diet:	Rat and Mouse No.1 maintenance diet, Special Diet Services, Stepfield, Witham, Essex, UK. *Ad libitum*
Water:	Tap water *ad libitum*
Environmental conditions:	Temperature: 21 ± 2°C
	Humidity: 40 - 70%
	Air changes: At least 15 changes/hour
	Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

Experimental Dates: Start: 24 April 2009 End: 04 September 2009

Group Arrangements: Animals were assigned to 2 groups as shown in the table below.

Table B.6.1.1/02/02-1: Dosing groups for pharmacokinetic studies for [^{14}C]-SYN545192

Test Group	Dose (mg/kg)	Number/sex	Remarks
Group 1 Single oral dose (low dose)	1	4 males, 4 females	Excreta collection over 168 hours and selected tissues taken for analysis.
Group 2 Single oral dose (high dose)	40	4 males, 4 females	Excreta collection over 168 hours and selected tissues taken for analysis.

Dosing and sample collection:

A single oral dose of [^{14}C]-SYN545192 suspended in CMC containing Tween 80 was administered to each rat by gavage in a dose volume of 10.7 and 10 mL/kg, for low and high doses, respectively. Animals in group 1 received a dose corresponding to a low dose of 1 mg/kg, and animals in group 2 received a dose corresponding to a high dose of 40 mg/kg. For both doses, the animals received a target radioactive dose of 5 MBq/kg.

Urine and faeces were separately collected from individual rats. Urine and faeces were frozen immediately upon collection. At the end of each faeces collection period, cage wash samples were collected (water). Expired air was collected into monoethanolamine:ethanediol (3:9) traps for 2 male and 2 female from each group.

Animals were humanely killed by CO_2 narcosis at 168 hours post dose. A terminal blood sample was taken and divided between two heparinised tubes, one of which was centrifuged to separate plasma. The following tissues were taken for radioactivity analysis: adrenals, brain, heart, kidneys, liver, lungs, ovaries (females), pancreas, spleen, testes (males), thymus, thyroid, uterus (females), gastrointestinal tract plus contents and residual carcasses together with representative samples of bone mineral, fat (renal), and muscle.

All samples were analysed for radioactivity by liquid scintillation counting either directly or following sample oxidation.

Excretion studies:

Urine was collected at intervals of 12, 24, 48, 72, 96, 120, 144 and 168 hours after dosing. Faeces and cage wash were collected at intervals of 24, 48, 72, 96, 120, 144 and 168 hours after dosing. Expired air was collected at intervals of 24 and 48 hours after dosing.

To investigate pharmacokinetics, terminal blood samples were collected via the vena cava or by cardiac puncture at 168 hours after dosing.

Metabolite characterisation studies: Metabolite characterisation on selected plasma samples was undertaken in a separate study (Green, M and MacDonald, M 2011) SYN545192 - Investigation of the Nature and Identity of Radiolabelled Metabolites Present in Urine, Faeces, Bile and Plasma Collected from Rats Following Oral Administration of [14C]-SYN545192. Charles River Report No. 31096. Unpublished. Syngenta File no. SYN545192_10131; Annex B.6.1.1/06.

Statistics: Not applicable.

RESULTS AND DISCUSSION

Excretion: In the single low dose experiment, the largest amounts of recovered radioactivity were excreted in the faeces in males and females with the largest amount of that being excreted in the first 48 hours. Urinary excretion was predominately within the first 12 hours in males and females.

In the single high dose experiments, again the largest amounts of recovered radioactivity were excreted in the faeces. In males, the excretion was predominately in the first 48 hours. In females, the faecal excretion was predominately in the first 72 hours and the two largest percentages were in the 24 – 72 hour range. Urinary excretion was predominately in the first 24 hours in males and in the first 72 hours in females.

Table B.6.1.1/02-2: Recovery of radioactivity in excreta and tissues after administration of a single oral dose of [^{14}C]-SYN545192 to rats

		Group mean excretion data (percentage of radioactive dose recovered)			
		Group 1 1mg/kg		Group 2 40 mg/kg	
		Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Urine	0-12 h	6.1 ± 1.8	2.5 ± 0.7	2.3 ± 0.5	0.9 ± 0.2
	12-24 h	3.6 ± 1.7	1.8 ± 0.3	2.4 ± 0.3	1.1 ± 0.4
	24-48 h	1.7 ± 0.3	1.2 ± 0.1	1.3 ± 0.6	3.0 ± 1.1
	48-72 h	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	1.3 ± 0.3
	72-96 h	0.1 ± <0.1	0.2 ± 0.1	0.1 ± <0.1	0.3 ± 0.3
	96-120 h	0.1 ± <0.1	0.1 ± <0.1	0.1 ± <0.1	0.1 ± <0.1
	120-144 h	0.1 ± <0.1	<0.1 ± <0.1	<0.1 ± <0.1	0.1 ± <0.1
	144-168h	0.1 ± <0.1	<0.1 ± <0.1	<0.1 ± <0.1	<0.1 ± <0.1
	Subtotal	12.2 ± 2.7	6.3 ± 0.9	6.5 ± 1.0	6.8 ± 1.6
Faeces	0-24 h	39.6 ± 8.1	40.5 ± 7.8	39.4 ± 9.2	16.9 ± 2.0
	24-48 h	34.3 ± 10.5	31.1 ± 12.9	42.7 ± 5.3	39.1 ± 5.5
	48-72 h	6.8 ± 2.0	13.2 ± 3.3	8.2 ± 3.3	25.6 ± 7.7
	72-96 h	2.3 ± 1.6	4.0 ± 2.3	1.6 ± 0.9	6.0 ± 3.5
	96-120 h	0.6 ± 0.2	1.1 ± 0.6	0.4 ± 0.2	2.0 ± 1.9
	120-144 h	0.3 ± 0.1	0.4 ± 0.2	0.2 ± <0.1	0.4 ± 0.2
	144-168h	0.2 ± <0.1	0.2 ± <0.1	0.2 ± <0.1	0.3 ± 0.1
	Subtotal	84.1 ± 2.3	90.4 ± 1.7	92.7 ± 2.6	90.3 ± 4.1
Cage wash		2.6 ± 1.4	0.6 ± 0.1	0.8 ± 0.1	3.0 ± 1.9
Expired Air		<0.1	<0.1	<0.1	<0.1
GI tract + contents		0.1 ± <0.1	0.1 ± <0.1	0.1 ± <0.1	0.1 ± <0.1
Tissues + carcass		1.8 ± 0.2	1.0 ± ~0.3	1.4 ± ~0.4	0.8 ± ~0.3

Total Recovery	100.9 ± 1.4	98.4 ± 1.8	101.6 ± 1.5	101.1 ± 0.3

Tissue distribution:

Residual radioactivity was expressed as µg equivalents of SYN545192/g of tissue and percentage of administered dose. The latter is presented in Table B.6.1.1/02-3.

In low- and high-dose males, the highest concentrations were found in the liver and G.I. contents, followed by the kidneys and G.I. tract. The percentage of administered dose retained in the tissues was unaffected by dose. In low-dose females, the highest concentrations were found in the G.I. contents, followed by the liver, G.I. tract and kidneys. In high-dose females, the highest concentrations were found in the liver, G.I. contents, G.I. tract and kidneys. Other than the G.I. contents, the percentage of administered dose retained was unaffected by dose.

Small amounts of retained radioactivity were found to be widely distributed among the remaining tissues.

Table B.6.1.1/02-3: Distribution of radioactivity tissues/organs 168 hours after administration of a single oral dose of [^{14}C]-SYN545192 to rats

Tissue/organ	Percent of administered dose (%)			
	Group 1 1 mg/kg		Group 2 40 mg/kg	
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Adrenals	0.0004 ± 0.0001	0.0004 ± <0.0001	0.0004 ± 0.0001	<0.0006 ± NA
Bone Mineral	0.0003 ± 0.0001	<0.0001 ± NA	<0.0002 ± NA	<0.0001 ± NA
Brain	0.0053 ± 0.0006	0.0015 ± 0.0002	0.0044 ± 0.001	0.0029 ± 0.0008
Fat- renal	0.0006 ± 0.0002	0.0007 ± 0.0001	0.0009 ± 0.0003	0.0014 ± 0.0004
G.I. Tract	0.0374 ± 0.0033	0.0259 ± 0.0070	0.0275 ± 0.0038	0.0298 ± 0.0132
G.I. Tract contents	0.0901 ± 0.0110	0.0813 ± 0.0413	0.0781 ± 0.0227	0.0695 ± 0.0434
Heart	0.0123 ± 0.0009	0.0037 ± 0.0007	0.0081 ± 0.0009	0.0056 ± 0.0013
Kidneys	0.0431 ± 0.0098	0.0132 ± 0.0007	0.0297 ± 0.0056	0.0180 ± 0.0069
Liver	0.2024 ± 0.0403	0.0661 ± 0.0122	0.1610 ± 0.0217	0.0895 ± 0.0233
Lungs	0.0138 ± 0.0029	0.0043 ± 0.0017	0.0099 ± 0.0013	0.0051 ± 0.0017
Muscle	0.0012 ± 0.0003	0.0005 ± 0.0003	0.0013 ± 0.0005	0.0010 ± 0.0006
Ovaries	NA	0.0002 ± 0.0001	NA	0.0005 ± 0.0003

Pancreas	0.0051 ± 0.0008	0.0018 ± 0.0005	0.0043 ± 0.0014	0.0034 ± 0.0008
Plasma	0.0032 ± 0.0021	0.0004 ± 0.0001	0.0014 ± 0.0006	0.0005 ± 0.0004
Residual Carcass	1.5410 ± 0.1188	0.9200 ± 0.3313	1.2288 ± 0.3618	0.7183 ± 0.2825
Spleen	0.0041 ± 0.0008	0.0011 ± 0.0004	0.0030 ± 0.0002	0.0016 ± 0.0006
Testes	0.0112 ± 0.0037	NA	0.0069 ± 0.0008	NA
Thymus	0.023 ± 0.0011	0.0011 ± 0.0007	0.0020 ± 0.0014	0.0014 ± 0.0006
Thyroid	0.0001 ± <0.0001	0.0001 ± <0.0001	0.0002 ± 0.0001	<0.0001 ± NA
Uterus	NA	0.0015 ± 0.0010	NA	0.0010 ± 0.0004
Whole Blood	0.0024 ± 0.0007	0.0005 ± 0.0001	0.0012 ± 0.0003	0.0008 ± 0.0003
Tissues + Carcass	1.9685 ± 0.1777	1.1222 ± 0.3356	1.5694 ± 0.3972	0.9472 ± 0.3588

NA = Not applicable

INVESTIGATORS' CONCLUSION:

Irrespective of dose or sex, a single oral dose of 1 or 40 mg [pyrazole-5-^{14}C]-SYN545192/kg was rapidly and extensively eliminated with the predominant route of elimination being *via* the faeces.

At both doses, residues of radioactivity were relatively low in blood and tissues by 7 days post dose, but remained detectable in both sexes. Tissue distribution was also similar in both sexes at both doses, with the highest concentrations in the organs of excretion (kidneys and liver). These findings were consistent with the extensive excretion of the administered dose.

REVIEWER'S CONCLUSION:

The reviewer agrees with the investigators' conclusions that single oral doses of 1 or 40 mg [pyrazole-5-^{14}C]-SYN545192/kg were rapidly and extensively eliminated, predominantly *via* the faeces.

Dose had little effect on the distribution of radioactivity or its elimination and sex differences were minimal. Relatively higher concentrations found in the G.I. tract, G.I. contents, liver and kidneys were correlated with the extensive excretion of SYN545192.

Report:	IIA 5.1.1/04. Shaw J, 2011d. SYN545192 – The Pharmacokinetics of [Pyrazole-^{14}C]-SYN545192 the Rat following a Single Oral Administration. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Charles River Report No. 30584. Issue date 10 August 2011. Unpublished (Syngenta File No. SYN545192_10121).EPA MRID No.48604426

Guidelines: Metabolism – rat; OECD 417 (1984); EPA OPPTS 870.7485 (1998); 87/302/EEC (1987), B36, 94/79/EC (1994), JMAFF 12 Nohsan No 8147(2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

This study was designed to determine the pharmacokinetics of SYN545192 and related material in the rat following the single oral administration of [pyrazole-5-^{14}C]-SYN545192 at doses of 1 mg/kg and 40 mg/kg to Han Wistar rats, and to collect excreta samples for total radioactivity analysis.

The majority of the radioactivity administered via single oral doses of 1 or 40 mg [pyrazole-5-^{14}C]-SYN545192/kg was excreted *via* the faeces in both males and females. In low-dose males and females and high-dose males, the majority of the faecal excretion occurred in the first 48 hours. In the high-dose females, the faecal excretion extended to 72 hours. Faecal excretion comprised 79 – 89% of the administered radioactivity. Urinary excretion was primarily in the first 24 hours in low-dose males and females and high-dose males and in the first 28 hours in high-dose females. Total urinary excretion was between 4.9 – 8.9% of the administered dose. Overall, excretion was extensive and between 90.6 – 96.1% of the administered dose was recovered. There were no large sex differences.

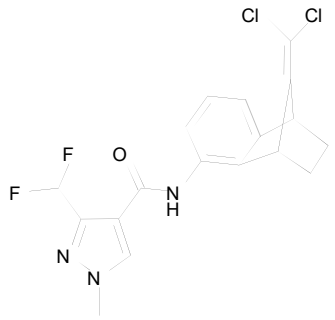
C_{max} and AUC values were higher in males than in females at equivalent doses, while T_{max} values were equivalent in low-dose animals (4h) and longer in high-dose females (24h) than in high-dose males (6h). Half-lives were roughly the same in low-dose females and high-dose males and females (26.9-34.3h) and longer in low-dose males (61.7h). Measurements of exposure (C_{max} and AUC values) were 15-20X higher in high-dose males than low-dose males. In females, the values were 22-36X higher in high-dose females than low-dose females. This would indicate a saturation in males that occurs at much higher doses in females. There were slight differences in whole blood and plasma measurements.

MATERIALS AND METHODS

Materials:

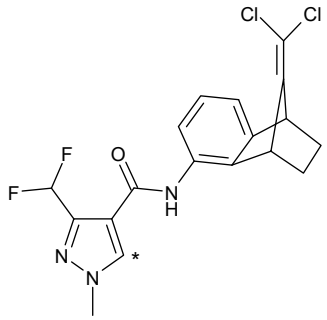
Unlabelled Test Material:	SYN545192
Physical state:	Beige solid
Purity:	97%
Source:	Syngenta Crop Protection Inc.
Lot/Batch number:	SMU9BP005

Structure:



Unlabelled test item was used in the high dose preparations to dilute the specific activity of the radiolabelled test item.

Radiolabelled Test Material	[Pyrazole-5-^{14}C]-SYN545192
Specific activity:	Low Dose: 5.46 MBq/mg High Dose: 0.125 and 0.118 MBq/mg
Radiochemical purity:	>98%
Source:	Selcia
Lot/Batch number:	5072PJS001-2
Structure:	



* position of [^{14}C]-label

Vehicle: 1% carboxymethylcellulose (CMC) containing 0.1% (v/v) Tween 80.

Preparation of dosing solutions: Radiolabelled SYN545192 was homogenously suspended in 1% CMC containing 0.1% (v/v) Tween 80 for dosing.

Test Animals:

Species:	Rat
Strain:	Han Wistar
Age/weight at dosing:	7-8 weeks Weight range at time of dosing: Low dose: 187– 259g (males), 147 – 192g (females) High dose: 199– 218g (males), 143 – 172g (females)
Source:	Charles River (UK) Limited
Housing:	During the pre-study holding period, rats were multiply housed (where possible) by sex in solid floored polycarbonate and stainless steel caging with bedding. Following dosing animals were housed in groups of 2 or 3 in polycarbonate and stainless steel cages with raised wire mesh floors. The rats used for excretion studies were housed singly in all-glass metabolism cages specially designed for the separate quantitative collection of urine and faeces.
Acclimatisation period:	5 days
Diet:	Rat and Mouse No.1 maintenance diet, Special Diet Services, Stepfield, Witham, Essex, UK. *.Ad libitum*.
Water:	Tap water *ad libitum*
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 - 70% Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

Experimental dates: Start: 21 April 2009; End: 23 July 2009

Group Arrangements: Animals were assigned to 4 groups as shown in the table below.

Table B.6.1.1/04-1: Dosing groups for pharmacokinetic studies for [^{14}C]-SYN545192

Test Group	Dose (mg/kg)	Number/sex	Remarks
Group 1 Blood and plasma collection, Excretion kinetics (low dose)	1	9 males, 9 females	Serial blood collections over a time course. Final sample taken as terminal. 3M & 3F also used for excreta collections over 5 days
Group 2 Blood and plasma collection, Excretion kinetics (high dose)	40	9 males, 9 females	Serial blood collections over a time course. Final sample taken as terminal. 3M & 3F also used for excreta collections over 5 days
Group 3 Blood and plasma collection (low dose)	1	2 males, 2 females	Terminal blood collections at T_{max}. Plasma provision for plasma metabolite profiling
Group 4 Blood and plasma collection (high dose)	40	2 males, 2 females	Terminal blood collections at $T_{max.}$ Plasma provision for plasma metabolite profiling

Dosing and sample collection: A single oral dose of [^{14}C]-SYN545192 suspended in CMC containing Tween 80 was administered to each rat by gavage in a dose volume of 10 mL/kg. Animals in groups 1 and 3 received a dose corresponding to a nominal low dose of 1 mg/kg, and animals in groups 2 and 4 received a dose corresponding to a nominal high dose of 40 mg/kg. For both doses, the animals received a target radioactive dose of 5 MBq/kg.

Urine and faeces were separately collected from 3 males and 3 females in groups 1 and 2. Urine and faeces were frozen immediately upon collection. At each collection period, cage wash samples were collected.

Serial blood samples were removed at 4 alternate time points per group of 3 male and 3 female animals by venepuncture of a tail vein. The last time point from each group was taken as terminal sample. Blood was collected into heparinised tubes. A sample of blood was retained and plasma was then separated from the remaining blood sample by centrifugation. Blood cells were discarded.

Animals used for excreta collection were terminated by CO_2 narcosis at 120 hours post-dose. A terminal blood sample was collected and divided between two heparinised tubes. One portion was retained for whole blood analysis and the remainder centrifuged to separate plasma. All samples were analysed for radioactivity by liquid scintillation counting either directly or following sample oxidation.

Pharmacokinetic studies:

To investigate pharmacokinetics, serial blood samples were collected by tail vein bleeding at the following intervals:

Table B.6.1.1/04-2: Blood collection times for pharmacokinetic studies for [^{14}C]-SYN545192

Groups	Nominal dose	Animal numbers		Sampling times (hours after dosing)
		Male	Female	
1	1 mg/kg	1-3 7-9 13-15	4-6 10-12 16-18	1, 6, 24 and 72[A] 2, 8, 36, 96[A] 4, 12, 48, 120[A]
2	40 mg/kg	19-21 25-27 31-33	22-24 28-30 34-36	1, 6, 24 and 72[A] 2, 8, 36, 96[A] 4, 12, 48, 120[A]

A = sample taken as terminal sample

Urine and faeces were collected from 3 male and 3 female rats from groups 1 and 2 at intervals of 0-24, 24-48, 48-72, 72-96 and 96-120 hours after dosing.

Metabolite characterisation studies: Metabolite characterisation on selected plasma samples was undertaken in a separate study (Green, M and MacDonald, M 2011) SYN545192 - Investigation of the Nature and Identity of Radiolabelled Metabolites Present in Urine, Faeces, Bile and Plasma Collected from Rats Following Oral Administration of [^{14}C]-SYN545192. Charles River Report No. 31096. Unpublished. Syngenta File no. SYN545192_10131, ***Annex B.6.1.1/06***.

Statistics: Not applicable.

RESULTS AND DISCUSSION

Pharmacokinetic Studies:

Excretion: The recovery of radioactivity in excreta following administration of a single oral dose of [^{14}C]-SYN545192 at nominal doses of 1 mg/kg and 40 mg/kg is presented in the table below.

Table B.6.1.1/04-3: Recovery of radioactivity in excreta and tissues after administration of a single oral dose of [^{14}C]-SYN545192 to rats

		Group mean excretion data (percentage of radioactive dose recovered)			
		Group 1		Group 2	
		1 mg/kg		40 mg/kg	
		Male (n=3)	Female (n=3)	Male (n=3)	Female (n=3)
Urine	0-24 h	7.0	3.1	3.5	2.4
	24-48 h	1.3	1.3	1.1	3.1
	48-72 h	0.3	0.4	0.2	0.6
	72-96 h	0.2	0.1	0.1	0.2
	96-120 h	0.1	0.1	<0.1	0.1
	Subtotal	8.9	5.1	4.9	6.4
Faeces	0-24 h	37.3	39.3	41.4	13.4
	24-48 h	36.5	33.1	40.9	48.7
	48-72 h	3.9	6.2	5.2	20.6
	72-96 h	0.8	2.7	1.0	3.8
	96-120 h	0.4	0.9	0.3	0.9
	Subtotal	78.9	82.3	88.8	87.6
Cage wash		1.4	1.1	0.8	0.8
GI tract + contents		0.2	0.5	0.1	0.3
Carcass		2.7	1.6	1.3	0.1
Total Recovery		92.2	90.6	95.9	96.1

As determined in the excretion and tissue distribution study, the majority of the radioactivity was excreted in the faeces within the first 48 hours following treatment. Urinary excretion comprised a much smaller part, but was primarily in the first 24 hours, with the exception of high-dose females, where urinary excretion took place over the first 48 hours. Excretion was extensive and accounted for 90.6 – 96.1% of the administered

dose. Small amounts of the administered dose were noted in the carcass up to 120 hours following administration.

Blood kinetics: The concentration of total radioactivity in plasma and whole blood following a single oral administration of [^{14}C]-SYN545192 at nominal doses of 1 mg/kg and 40 mg/kg are presented in the tables below.

Table B.6.1.1/04-4: Concentrations of radioactivity in whole blood over a time course after administration of [^{14}C]-SYN545192

Sampling time	Group mean blood concentrations (µg equivalents of [^{14}C]-SYN545192/g)			
	Group 1		Group 2	
	1 mg/kg		40 mg/kg	
	Male (n=3)	Female (n=3)	Male (n=3)	Female (n=3)
1 h	0.133	0.108	1.9	1.3
2 h	0.196	0.143	2.3	1.4
4 h	0.272	0.146	2.4	1.7
6 h	0.227	0.146	4.3[1]	2.8[2]
8 h	0.221	0.122	3.8	2.0[3]
12 h	0.235	0.113	4.0	3.0
24 h	0.125	0.071	4.0	3.2
36 h	0.096	0.050	2.8	2.3
48 h	0.107	0.033	1.5	1.9
72 h	0.060	0.022	1.4	1.0
96 h	0.044	0.010	0.8	0.3
120 h	0.048	0.006	0.5	0.4

[1] = Mean of two animals only, no blood sample obtained for animal 20M at 6 hours post dose

[2] = Mean of two animals only, no blood sample obtained for animal 23F at 6 hours post dose

[3] = Mean of two animals only, no blood sample obtained for animal 28F at 8 hours post dose

Table B.6.1.1/04-5: Concentrations of radioactivity in plasma over a time course after administration of [^{14}C]-SYN545192

Sampling time	Group mean plasma concentrations (µg equivalents of [^{14}C]-SYN545192/g)			
	Group 1		Group 2	
	1 mg/kg		40 mg/kg	
	Male (n=3)	Female (n=3)	Male (n=3)	Female (n=3)
1 h	0.165	0.112	2.0	1.5
2 h	0.238	0.133	2.8	1.5
4 h	0.376	0.129	2.7	1.6
6 h	0.312	0.120	5.4[1]	1.9[2]
8 h	0.286	0.107	4.6	2.0[3]
12 h	0.366	0.091	4.8	2.4
24 h	0.199	0.060	4.9	3.1

Sampling time	Group mean plasma concentrations (µg equivalents of [^{14}C]-SYN545192/g)			
	Group 1		Group 2	
	1 mg/kg		40 mg/kg	
	Male (n=3)	Female (n=3)	Male (n=3)	Female (n=3)
36 h	0.149	0.047	3.3	2.0
48 h	0.162	0.027	1.9	2.0
72 h	0.088	0.019	2.0	1.1
96 h	0.061	0.010	1.2	0.4
120 h	0.068	0.006	0.7	0.5

[1] = mean of two animals only, no blood sample obtained for animal 20M at 6 hours post dose

[2] = Mean of two animals only, no blood sample obtained for animal 23F at 6 hours post dose

[3] = Mean of two animals only, no blood sample obtained for animal 28F at 8 hours post dose

Table B.6.1.1/04-6: A comparison of pharmacokinetic parameters in blood following administration of [^{14}C]-SYN545192

	Pharmacokinetic parameters			
	Group 1 1 mg/kg		Group 2 40 mg/kg	
	Male	Female	Male	Female
C_{max} (µg equiv/g)	0.272	0.146	4.32	3.24
T_{max} (h)	4	4	6	24
$t_{1/2}$ term (h)	61.7	26.9	34.3	27.8
AUC_0-t (µg equiv .h/mL)	11.6	5.06	231.4	180.2
AUC_0-inf (µg equiv .h/mL)	15.9	5.29	258.3	194.9

Table B.6.1.1/04-7: A comparison of pharmacokinetic parameters in plasma following administration of [^{14}C]-SYN545192

	Toxicokinetic parameters			
	Group 1 1 mg/kg		Group 2 40 mg/kg	
	Male	Female	Male	Female
C_{max} (µg equiv/mL)	0.376	0.133	5.42	3.06
T_{max} (h)	4	2	6	24
$t_{1/2}$ term (h)	55.2	28.5	29.8	33.1
AUC_0-t (µg equiv .h/mL)	17.2	4.40	292	174
AUC_0-inf (µg equiv .h/mL)	22.6	4.63	320	197

The T_{max} in low- and high-dose males and high-dose females was consistent in blood and plasma at 4, 6 and 24 hours, respectively. In low-dose females, T_{max} was 4 hours in blood and 2 hours in plasma. The C_{max} was highest in high-dose males and lowest in

low-dose females. The area under the curve was 3-5X higher in low-dose males than in low-dose females. It was 1.3-1.6X higher in high-dose males than in high-dose females. It was 14-16X higher in high-dose males than low-dose males and 37-43% higher in high-dose females than low-dose females.

INVESTGATORS' CONCLUSION:

Following a single oral dose of [pyrazole-5-^{14}C]-SYN545192/kg to male and female rats, the mean peak plasma concentrations were reached after approximately 2-4 hours and 6-24 hours following doses of 1 mg/kg and 40 mg/kg, respectively. Systemic exposure to total radioactivity was comparable between plasma and whole blood.

Systemic exposure to total radioactivity appeared greater in males compared with females. There appeared to be a trend towards broad dose proportionality in AUC estimates in females, however all other increases were generally less than proportional with respect to increase in dose.

REVIEWER'S CONCLUSIONS:

When given single oral doses of [pyrazole-5-^{14}C]-SYN545192, uptake was rapid. Excretion was rapid as well, with the majority excreted in the faeces within 48 hours and a minority in the urine. Excretion was extensive and over 90% regardless of dose or sex, but there was still radioactivity left in the tissues after 120 hours.

T_{max} was 4 – 6 hours in males in low- and high-doses, respectively. In females, T_{max} was 2 hours in plasma and 4 hours in blood in low-dose females, while it was 24 hours in high-dose females.

B.6.3 Short-term toxicity

B.6.3.1 ORAL 28-DAY TOXICITY (RODENTS)

Study Type: SYN545192 – Twenty-Eight Day Repeated Oral (Dietary) Toxicity Study in the Rat; EPA OPPTS 870.3050

Test Material (purity): Not reported

Citation: SYN545192 – Twenty-Eight Day Repeated Oral (Dietary) Toxicity Study in the Rat. Harlan Laboratories Limited Shardlow, UK. Laboratory report number: 2364/0198. Study completion date: January 4, 2010.

Sponsor: Syngenta Crop Protection, LLC, USA.

Executive Summary:

The purpose of this study was to identify any treatment-related effects following continuous oral (dietary) administration of test material for up to 28 days.

The test material was administered continuously throughout the treatment period by dietary admixture on a parts per million (ppm) basis to three groups, each of five male and five female Wistar HanTM: HsdRccHanTM: WIST strain rats, for twenty-eight consecutive days, at dietary concentrations of 100, 400 and 1200 ppm (equivalent to a mean achieved dosage of 9, 36 and 99 mg/kg/day). A control group of five males and five females were treated with basal laboratory diet alone.

There were no unscheduled deaths during the study and no adverse clinical signs were observed. There were no treatment-related changes in the behavioural or sensory reactivity assessments and no changes in the functional performance parameters measured. There was a reduction in body weight gain and food consumption in those animals dosed at 1200 ppm.

There were no treatment-related changes to the haematology or clinical chemistry parameters measured. Increases in liver and heart weights were evident in males treated at 1200 ppm.

Oral (dietary) administration of SYN545192 to rats for a period of twenty-eight days resulted in a toxicologically significant reduction in body weight and body weight gain at 1200 ppm.
Minimal tubular basophilia was observed in the kidneys of females at 400 and 1200 ppm.
Minimal centrilobular hypertrophy was observed in males at 400 and 1200 ppm.

A clear “No Observed Effect Level” (NOEL) was established at 100 ppm, equivalent to 9 mg/kg/day in both males and females.

EPA Conclusion: LOAEL = 99 mg/kg/day based on decreased body weight, hypoglycaemia, and decreased serum potassium. NOAEL = 36 mg/kg/day.

Materials and Methods

Materials

Test material:	SYN545192	
Description:	white powder	
Lot/Batch #:	TE-6341/7	
Purity:	Not reported	
Vehicle:	Not reported	
Positive control:	Not reported	
Test species:	Rats	
Strain:	Wistar HanTM: HsdRccHanTM:	
Sex:	male and female	
Age at start:	thirteen weeks old	
Weight at start:	males weighed 175 to 198g; females weighed 131 to 158g	
Source:	Harlan Laboratories UK Ltd	
Housing:	The animals were housed in groups of five by sex, in solid floor polypropylene cages with stainless steel mesh lids and furnished with softwood flakes	
Diet:	The animals were allowed free access to food	
Water:	The animals were allowed free access to water.	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	21 ± 2ºC 55 ± 15% fifteen air changes per hour twelve hours continuous light and twelve hours darkness
Acclimation:	Not reported	

Study design and methods

Study experimentation dates - Start: 24 December 2007 End: January 4, 2010

Diet preparation and analysis - For the purpose of this study, the test material was prepared at the appropriate concentrations as a dietary admixture in SQC Rat and Mouse Ground Diet No.1 (special diet services limited, Witham, Essex, UK). The stability and homogeneity of the test material in the diet were not determined by Harlan Laboratories Ltd, Shardlow, UK Analytical Laboratory.

The test material was administered continuously, for twenty-eight consecutive days, by dietary admixture. The achieved doses were calculated to be equivalent to a mean achieved dosage of 9, 36 and 107 mg/kg bw/day for males and 9, 36 and 90 mg/kg bw/day for females. Control animals were treated in an identical manner with basal laboratory diet.

Animal assignment – Animals were allocated to treatment groups as follows:

Table 1. Study design

Treatment Group	Dietary Concentration (ppm)	Animal Numbers	
		Male	Female
Control	0	5 (1-5)	5 (6-10)*
Low	100	5 (11-15)	5 (16-20)
Intermediate	400	5 (21-25)	5 (26-30)
High	1200	5 (31-35)	5 (36-40)

*The numbers in parentheses ( ) show the individual animal numbers allocated to each treatment group.

Clinical observations

All animals were examined for overt signs of toxicity, ill-health or behavioural change once daily. All observations were recorded.

Functional observations

Motor Activity - Twenty purposes built 44 infra-red beam automated activity monitors were used to assess motor activity. Animals of one sex were tested at each occasion and were randomly allocated to the activity monitors. The tests were performed at approximately the same time each day, under similar laboratory conditions.

Forelimb/Hindlimb Grip Strength - An automated grip strength meter was used. Each animal was allowed to grip the proximal metal bar of the meter with its forepaws. The animal was pulled by the base of the tail until its grip was broken. The animal was drawn along the trough of the meter by the tail until its hind paws gripped the distal metal bar.

Sensory reactivity - Each animal was individually assessed for sensory reactivity to auditory, visual and proprioceptive stimuli. The following parameters were observed:

- Grasp response Touch escape
- Vocalisation Pupil reflex
- Toe pinch Startle reflex
- Tail pinch Blink reflex
- Finger approach

Body weight

Individual body weights were recorded twice weekly throughout the acclimatisation period, daily during Week 1 of treatment and then twice weekly for the duration of the study. Individual body weights were also recorded at terminal kill.

Food consumption and Water consumption

Food consumption was recorded twice weekly throughout the acclimatisation period, daily during Week 1 of treatment and then twice weekly for the duration of the study. Group dietary intake and weekly food efficiency (body weight gain/food intake) was calculated for each sex.

Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.

Haematology

The following parameters were measured on blood collected into tubes containing potassium EDTA anti-coagulant:
- Haemoglobin (Hb)
- Erythrocyte count (RBC)
- Haematocrit (Hct)
- Erythrocyte indices:
  - mean corpuscular haemoglobin (MCH)
  - mean corpuscular volume (MCV)
  - mean corpuscular haemoglobin concentration (MCHC)

Total leucocyte count (WBC)

Differential leucocyte count:
- neutrophils (Neut)
- lymphocytes (Lymph)
- monocytes (Mono)
- eosinophils (Eos)
- basophils (Bas)

Platelet count (PLT)

Reticulocyte count (Retic)
- Cresyl blue stained slides were prepared but reticulocytes were not assessed

Prothrombin time (CT) was assessed by 'Inovin' and Activated partial thromboplastin time (APTT) was assessed by 'Actin FS' using samples collected into sodium citrate solution (0.11 mol/l).

Blood chemistry
The following parameters were measured on plasma from blood collected into tubes containing lithium heparin anti-coagulant:
- Urea Inorganic phosphorus (P)
- Glucose Aspartate aminotransferase (ASAT)
- Total protein (Tot.Prot.) Alanine aminotransferase (ALAT)
- Albumin Alkaline phosphatase (AP)
- Albumin/Globulin (A/G) ratio (by calculation) Gamma glutamyltranspeptidase (γGT)
- Sodium (Na+) Creatinine (Creat)
- Potassium (K+) Total cholesterol (Chol)
- Chloride (Cl-)
- Calcium (Ca++)
- Total bilirubin (Bili)
- Triglycerides

Pathology
On completion of the treatment period, all animals were killed by intravenous overdose of sodium pentobarbitone followed by exsanguination. All animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded.

Organ weights
The following organs, removed from animals that were killed at the end of the study, were dissected free from fat and weighed before fixation:
- Adrenals
- Brain
- Epididymides

- Heart - Kidneys - Liver

- Ovaries - Spleen - Testes

- Thymus - Uterus/cervix - Macroscopic abnormalities

Histopathology

Samples of the following tissues were removed from all animals and preserved in buffered 10% formalin:

- Adrenals	- Aorta (thoracic)	- Bone & bone marrow (femur including stifle joint)	
- Bone & bone marrow (sternum)		**- Brain (including cerebrum, cerebellum and pons)**	
- Caecum	**- Colon**	**- Duodenum**	
- Epididymides ♦	- Eyes	**- Gross lesions**	
- Heart	**- Ileum (including peyer's patches)**		**- Jejunum**
- Kidneys	**- Liver**	**-Lungs (with bronchi) #**	
- Lymph nodes (cervical and mesenteric)		- Muscle (skeletal)	- Oesophagus
- Ovaries	- Pancreas	- Pituitary	- **Prostate**
- Rectum	**-** Salivary glands (submaxillary)		**- Sciatic nerve**
- Seminal vesicles	**-** Skin (hind limb)		**-Spinal cord**
- Spleen	**- Stomach**		**- Testes ♦**
- Thymus	**- Thyroid/parathyroid**	**- Trachea**	
- Urinary bladder	**- Uterus**		

♦ Preserved in Bouins fluid, then transferred to 70% Industrial Methylated Spirits (IMS) up to 48 hours later
Lungs were inflated to approximately normal inspiratory volume with buffered 10% formalin before immersion in fixative.

The tissues shown in bold from all control and 1200 ppm dose group animals were prepared as paraffin blocks, sectioned at nominal thickness of 5 µm and stained with haematoxylin and eosin for subsequent microscopic examination.

Evaluation of data

Data were processed to give group mean values and standard deviations where appropriate. Haematological, blood chemical, organ weight (absolute and relative to terminal body weight), weekly body weight gain and quantitative functional performance and sensory reactivity data were assessed for dose response relationships by linear regression analysis, followed by one way analysis of variance (ANOVA) incorporating Levene´s test for homogeneity of variance. Where variances were shown to be homogenous, pairwise comparisons were conducted using Dunnett´s test. Where Levene´s test showed unequal variances the data were analysed using non-parametric methods: Kruskal-Wallis ANOVA and Mann-Whitney U test.
The haematology variable basophils was not analysed since consistently greater than 30% of the data were recorded as the same value.
Probability values (p) are presented as follows:
$p < 0.01$ **
$p < 0.05$ *
p . 0.05 (not significant)

Results
Mortality

There were no unscheduled deaths during the study and no adverse clinical signs were observed.

Behavioural assessment

There were no treatment-related changes in the behavioural or sensory reactivity assessments and no changes in the functional performance parameters measured.

Functional Observations

There were no treatment-related changes in the behavioural or sensory reactivity assessments and no changes in the functional performance parameters measured.

Body Weight

Both males and females treated with 1200 ppm showed a reduction in body weight and body weight gain compared to control throughout the treatment period.

There were no effects on body weight in animals treated with 100 and 400 ppm. Occasional, statistically significant differences in body weight gain were small, isolated and showed no dose response relationship and are therefore considered not to be treatment related.

Table 2. Group Mean Body weight Gains

Group (Sex)	Base Weight Day 1	From To:	1 2	2 3	3 4	4 5	5 6	6 7	7 8	8 12	12 15	15 19	19 22	22 26	26 29	Abs Gain 1 29	% Gain 1 29
1 (F)	141.4	Mean	1.4	1.8	1.0	4.0	1.6	2.2	1.8	6.2	5.8	4.2	5.8	4.0	3.2	43.0	30.5
	9.5	S.D.	2.3	2.2	2.36	3.7	3.5	2.2	4.0	2.9	3.0	3.3	2.4	3.5	4.6	4.5	3.3
	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2 (F)	145.2	Mean	0.8	3.4	1.0	4.0	-2.8	3.6	2.8	7.8	3.0	6.4	5.4	4.0	3.2	42.6	29.3
	8.6	S.D.	2.6	2.3	3.4	1.2	3.7	3.7	4.0	1.9	3.0	1.8	2.9	1.2	1.5	8.6	5.5
	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3 (F)	142.2	Mean	-0.4	2.4	0.24	2.8	0.6	3.2	0.4	8.4	1.2[x]	5.0	4.2	4.4	1.4	36	25.1
	7.1	S.D.	1.5	2.7	3.6	2.2	2.3	4.0	1.1	2.1	2.2	1.0	3.8	2.6	2.1	12.9	8.4
	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4 (F)	142.8	Mean	-4.4**	-0.4	1.6	0.6	-1.8	3.4	-0.6	8.6	0.8*	4.6	1.6	5.0	1.0	20.0	4.2
	6.4	S.D.	1.8	3.3	2.6	2.7	2.2	1.5	2.9	5.9	3.4	2.0	4.1	3.0	3.5	9.3	7.0
	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1 (M)	188.2	Mean	6.2	4.2	3.6	7.0	4.6	5.6	7.2	19.0	14.8	16.6	14.0	14.2	10.4	127.4	67.6
	7.2	S.D.	1.3	1.6	2.3	2.0	1.8	2.4	0.8	3.5	2.2	5.2	2.9	5.5	2.6	22.2	11.0
	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2 (M)	187.4	Mean	5.8	4	5.6*	7	4.4	5.2	6.6	18.8	12.6	15	9.8	14	5.8	114.6	61.3
	8.7	S.D.	1.6	1	1.1	1.2	2.1	3	2.6	2.9	1.8	1.2	1.6	2.9	5.3	10.8	7.3
	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

3 (M)	187.2	Mean	5.8	4.4	5.4*	5.8	4.4	6.2	3.8	18	14	14.4	12.4	12.6	9.4	116.6	62.2
	7.4	S.D.	1.5	1.7	1.5	1.9	2.7	1.9	0.8	2.5	3.6	3.7	2.6	1.1	4.6	20.5	10
	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

4 (M)	187.2	Mean	-1.6*	5.2	5.8	6.6	1.8	3.6	5.0	15.6	10.8	12.6	10.6	9.6	7.6	93.2	50.0
	5.4	S.D.	1.8	2.4	0.8	1.1	2.1	3.6	2.1	2.6	3.8	4.4	3.4	4.4	1.1	21.7	12.2
	5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Dietary Concentrations: Group 1 - 0(Control) Group 2 - 100 ppm Group 3 - 400 ppm Group 4 - 1200 ppm

Food Consumption

All animals treated with 1200 ppm showed a reduction in food intake during Week 1 of treatment, with the reduction extending throughout the treatment period in females at 1200 ppm. Furthermore, animals of both sexes treated with 1200 ppm showed a reduction in food efficiency throughout the treatment period compared to the controls.
No effect on food intake or food efficiency was evident for animals treated with 100 or 400 ppm.

Haematology

There were no treatment-related changes to the haematology or clinical chemistry parameters measured.

Blood Chemistry

A statistically significant ($p<0.05$) reduction in plasma total protein levels and albumin levels was evident in all treated females. In addition, females treated with 1200 ppm displayed a slight but statistically significant ($p<0.05$) increase in plasma aspartate aminotransferase activity. A slight but statistically significant ($p<0.05$) reduction in plasma glucose levels was observed in males at 1200 ppm.
There were a number of statistically significant changes in plasma electrolyte levels in both sexes. However, the majority of the individual values were within normal ranges and in the absence of a convincing effect on electrolyte balance or any indication of a dose-related response, the intergroup differences were considered not to be treatment related.

Table 3. Group Mean Blood Chemical

Group (Sex)		Urea mg/dl	Gluco mg/dl	Tot Prote g/dl	Album g/dl	A/G Ratio	Na+ mmol/l	K+ mmol/l	Cl- mmol/l	Ca++ mmol/l	P mmol/l	yGT IU/l	ASAT IU/l	ALAT IU/l	AP IU/l	Creat mg/dl	Tri mg/dl	Chol mg/dl	Bili mg/dl
1 (F)	Mean	29.4	198.0	6.678	4.340	1.85	148.0	4.704	102.4	2.970	2.28	5.8	53.80	42.4	55.00	0.81	89.8	73.6	0.13
	S.D.	3.0	82.1	0.158	0.150	0.07	2.9	1.142	1.1	0.186	0.24	2.7	9.26	10.4	32.93	0.46	35.2	20.7	0.15
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2 (F)	Mean	29.6	162.4	6.13**	4.048*	1.95	148.2	3.900	104.0*	2.820*	2.20	4.6	56.60	34.0	55.80	0.80	105.2	66.4	0.08
	S.D.	3.2	8.2	0.201	0.127	0.12	1.3	0.570	1.4	0.085	0.40	3.1	8.38	9.30	13.97	0.02	22.2	16.7	0.11
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3 (F)	Mean	30.4	155.0	6.28**	4.082*	1.85	148.4	4.136	103.6*	2.812*	2.28	2.2	67.80	47.0	52.00	0.77	98.2	63.2	0.13
	S.D.	7.7	22.6	0.284	0.247	0.13	1.1	0.646	1.1	0.055	0.35	2.2	17.96	7.78	18.72	0.09	23.8	11.3	0.07
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

4 (F)	Mean	36.4	153.4	6.20**	3.996**	1.80	150.8*	4.388	104.4*	2.810*	1.94	5.8	81.0**	39.6	55.60	0.76	115.6	67	0.06
	S.D.	5.2	12.5	0.166	0.139	0.07	1.6	0.150	0.9	0.083	0.23	2.5	27.11	9.91	24.68	0.04	28.7	14.6	0.08
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1 (M)	Mean	31.8	163.8	5.674	3.556	1.68	148.8	4.948	101.4	2.806	2.62	5.4	60.00	63.4	146.4	0.74	110.4	56.2	0.05
	S.D.	5	17.6	0.307	0.162	0.14	1.1	0.438	1.1	0.134	0.37	0.8	13.49	14.1	51.8	0.1	46.6	10.6	0.07
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2 (M)	Mean	32.00	159.40	6.04	3.68	1.57	149.4	4.68	101.60	2.84	2.50	1.8	59.60	60.6	134.0	0.73	117.2	62.6	0.05
	S.D.	5.1	15.2	0.455	0.243	0.10	2.1	0.291	1.3	0.178	0.24	2.6	14.62	5.86	22.63	0.07	56.9	14.4	0.05
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3 (M)	Mean	37.00	168.00	5.92	3.61	1.57	148.0	4.394*	101.20	2.88	2.66	4.6	58.40	55.8	118.0	0.72	103.6	55.0	0.09
	S.D.	6.70	17.40	0.11	0.09	0.08	1.00	0.49	1.10	0.07	0.15	2.5	13.07	10.7	54.99	0.02	18.80	10.3	0.09
	N	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.00
4 (M)	Mean	30.8	138.6*	5.842	3.612	1.62	148.4	4.344*	102.2	2.796	2.30	5.4	67.60	59.0	123.0	0.76	113.4	62.0	0.15
	S.D.	6.1	7.2	0.393	0.222	0.12	1.3	0.291	1.5	0.088	0.29	2.7	7.64	9.82	31.92	0.14	42.0	4.0	0.14
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Dietary Concentrations: Group 1 - 0(Control) Group 2 - 100 ppm Group 3 - 400 ppm Group 4 - 1200 ppm

Histopathology

KIDNEY: Minimal tubular basophilia was evident in males treated with 100 (4/5) and 400 ppm (2/5). However given the absence of this change in the high dose group males, the lack of dose response at 100 and 400 ppm and the presence of a single incidence of this finding in control males, this finding are considered to be incidental to treatment. An increased incidence of minimal tubular basophilia was also observed in the treated female groups compared to controls with an apparent dose response. Given the observation of a single incidence of this finding in control males, the single incidence observed in 100 ppm females is considered unrelated to treatment.

LIVER: Minimal centrilobular hepatocyte hypertrophy was observed in the 400 and 1200 ppm males. No such effect was detected in for males treated with 100 ppm or female treated animals.

All other recorded histopatholgical changes were typical of the spontaneously arising background findings recorded in rats of this strain and ages used and were therefore considered not to be treatment related.

Table 4. Individual Necropsy Findings
Dose Level: 0 (Control)

Animal Number and Sex	Mode of Death	Tissue	Observation
1 M	Terminal kill	Liver	Inflammatory cell infiltrate +/- necrosis (minimal)

2 M	Terminal kill	Heart Kidneys Liver Parathyroid Prostate	Myocarditis/myofibre degeneration, subepicardial (slight) Interstitial mononuclear cell infiltration, focal, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis (minimal) One present Inflammatory cell infiltration (minimal)
3 M	Terminal kill	Kidneys Liver Prostate	Interstitial mononuclear cell infiltration, focal,unilateral (minimal) Tubular basophilia, focal, unilateral (minimal) Liver Inflammatory cell infiltrate +/- necrosis (minimal) Inflammatory cell infiltration (minimal)
4 M	Terminal kill	Cervical lymph nodes Liver Parathyroid	Haemorrhage/congestion (minimal) Liver Inflammatory cell infiltrate +/- necrosis (minimal) One present
5 M	Terminal kill	Kidneys Liver Lungs Parathyroid	Interstitial mononuclear cell infiltration, focal unilateral (minimal) Liver Inflammatory cell infiltrate +/- necrosis (minimal) Alveolar histiocytosis, focal (minimal) One present
6 F	Terminal kill	Liver Parathyroid	Inflammatory cell infiltrate +/- necrosis (minimal) One present
7 F	Terminal kill	Liver Parathyroid	Inflammatory cell infiltrate +/- necrosis (minimal) One present
8 F	Terminal kill	Kidneys Liver Uterus	Interstitial mononuclear cell infiltration, focal, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis (minimal) Luminal dilation (slight)
9 F	Terminal kill	Liver	Inflammatory cell infiltrate +/- necrosis (minimal)
10 F	Terminal kill	Parathyroid	One present

Table 5. Individual Necropsy Findings
Dose Level: 100 ppm

Animal Number and Sex	Mode of Death	Tissue	Observation
11 M	Terminal kill	Kidneys Liver	Interstitial mononuclear cell infiltration (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
12 M	Terminal kill	Kidneys Liver	Interstitial mononuclear cell infiltration unilateral (minimal) Tubular basophilia (minimal) Inflammatory cell infiltrate +/- necrosis, focal (minimal)
13 M	Terminal kill	Kidneys Liver	Tubular basophilia, focal, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
14 M	Terminal kill	Kidneys Liver	Tubular basophilia, focal, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
15 M	Terminal kill	Kidneys Liver	Interstitial mononuclear cell infiltration (minimal) Tubular basophilia (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
16 F	Terminal kill	Kidneys Liver	Mineralisation, corticomedullary, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
17 F	Terminal kill	Kidneys Liver	Tubular basophilia, focal, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)

18 F	Terminal kill		No abnormalities detected
19 F	Terminal kill	Liver	Inflammatory cell infiltrate +/- necrosis (minimal)
20 F	Terminal kill	Kidneys Liver	Mineralisation, corticomedullary (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)

Table 6. Individual Necropsy Findings
Dose Level: 400 ppm

Animal Number and Sex	Mode of Death	Tissue	Observation
21 M	Terminal kill	Kidneys Liver	Interstitial mononuclear cell infiltration (minimal) Tubular basophilia (minimal) Centrilobular hypertrophy (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
22 M	Terminal kill	Kidneys Liver	Interstitial mononuclear cell infiltration unilateral (minimal) Tubular basophilia (minimal) Centrilobular hypertrophy (minimal)
23 M	Terminal kill	Kidneys Liver	Interstitial mononuclear cell infiltration, focal, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
24 M	Terminal kill	Kidneys Liver	Interstitial mononuclear cell infiltration, focal, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
25 M	Terminal kill	Kidneys Liver	Interstitial mononuclear cell infiltration, focal, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
26 F	Terminal kill	Kidneys Liver	Tubular basophilia, focal, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
27 F	Terminal kill	Kidneys Liver	Interstitial mononuclear cell infiltration (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
28 F	Terminal kill	Kidneys Liver	Interstitial mononuclear cell infiltration (minimal) Tubular basophilia, focal, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
29 F	Terminal kill	Liver	Inflammatory cell infiltrate +/- necrosis (minimal)
30 F	Terminal kill	Kidneys Liver	Interstitial mononuclear cell infiltration (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)

Table 7. Individual Necropsy Findings
Dose Level: 1200 ppm

Animal Number and Sex	Mode of Death	Tissue	Observation
31 M	Terminal kill	Liver	Centrilobular hypertrophy (minimal)

32 M	Terminal kill	Kidneys Liver	Interstitial mononuclear cell infiltration, focal, unilateral (minimal) Centrilobular hypertrophy (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
33 M	Terminal kill	Liver	Centrilobular hypertrophy (minimal) Inflammatory cell infiltrate +/- necrosis (minimal)
34 M	Terminal kill	Liver Parathyroid	Centrilobular hypertrophy (minimal) Inflammatory cell infiltrate +/- necrosis (minimal) One present
35 M	Terminal kill	Epididymides Liver Parathyroid	Inflammatory cell infiltration, unilateral (miminal) Centrilobular hypertrophy (minimal) Inflammatory cell infiltrate +/- necrosis (minimal) Inflammatory cell infiltration, unilateral (miminal)
36 F	Terminal kill	Cervical lymph nodes Kidneys	Not examined Tubular basophilia, focal, unilateral (minimal)
37 F	Terminal kill	Kidneys Liver Parathyroid Uterus	Tubular basophilia, focal, unilateral (minimal Inflammatory cell infiltrate +/- necrosis, focal (minimal) One present Luminal dilation (slight)
38 F	Terminal kill	Liver Parathyroid	Inflammatory cell infiltrate +/- necrosis (minimal) One present
39 F	Terminal kill	Kidneys Liver Parathyroid Uterus	Tubular basophilia, focal, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis, focal (minimal) One present Luminal dilation (slight)
40 F	Terminal kill	Kidneys Liver Lungs Parathyroid	Tubular basophilia, focal, unilateral (minimal) Inflammatory cell infiltrate +/- necrosis (minimal) Alveolar histiocytosis (minimal) Absent

Organ Weights

A statistically significant increase in absolute and relative liver ($p<0.01$) and heart ($p<0.05$) weights was observed in males at 1200 ppm. No such effect was observed for females treated with 1200 ppm or for any animals treated at 100 and 400 ppm (See table 8).

Table 8. Group Mean Organ Weights with Corresponding Relative (% of Body weight) Organ Weights

		MALES					FEMALES			
		0 Control	100 ppm	400 ppm	1200 ppm		0 Control	100 ppm	400 ppm	1200 ppm
Terminal Body weight	Mean (g)	315.6	302	303.8	280.4		184.4	187.8	178.2	162.8
	S.D.	26.9	12.1	25	19.4		11.4	14.9	18.5	6.4
	N	5	5	5	5		5	5	5	5
Adrenals	Mean (g)	0.0518	0.0511	0.047	0.0515		0.0610	0.0635	0.0589	0.0552
	S.D.	0.00712	0.00802	0.0121	0.00463		0.00837	0.00442	0.01533	0.01122
	N	5	5	5	5		5	5	5	5
Brain	Mean	1.91472	1.88224	1.8717	1.89688		1.74594	1.76186	1.7431	1.71878

(Including Cerebrum,	(g)								
	S.D.	0.15831	0.07887	0.1011	0.06592	0.10287	0.03073	0.12767	0.08164
	N	5	5	5	5	5	5	5	5
Epididymides	Mean (g)	1.02188	0.96032	1.0052	0.8995				
	S.D.	0.12135	0.6911	0.1523	0.06131				
	N	5	5	5	5				
Heart	Mean (g)	0.89236	0.92384	0.9096	0.91720*	0.60294	0.60874	0.62228	0.53946
	S.D.	0.12347	0.08299	0.1094	0.09756	0.08804	0.05055	0.05558	0.02127
	N	5	5	5	5	5	5	5	5
Kidneys	Mean (g)	1.7428	1.67514	1.6632	1.62898	1.05772	1.06424	1.05796	1.02692
	S.D.	0.16985	0.26644	0.2041	0.05918	0.13931	0.0704	0.15055	0.06441
	N	5	5	5	5	5	5	5	5
Liver	Mean (g)	11.022	10.6768	10.951	11.3377**	6.10612	5.99588	6.19758	6.15786
	S.D.	1.80704	0.77232	0.8798	0.70293	0.54081	0.70433	0.69379	0.44902
	N	5	5	5	5	5	5	5	5
Ovaries	Mean (g)					0.09494	0.09946	0.11038	0.1002
	S.D.					0.02319	0.01448	0.01682	0.02154
	N					5	5	5	5
Spleen	Mean (g)	0.6268	0.538	0.5828	0.4998	0.3776	0.39278	0.39708	0.31532
	S.D.	0.09114	0.09223	0.081	0.05081	0.06305	0.06734	0.06878	0.03817
	N	5	5	5	5	5	5	5	5
Testes	Mean (g)	3.1892	3.20554	3.2485	3.16728				
	S.D.	0.18659	0.13024	0.2852	0.2012				
	N	5	5	5	5				
Thymus	Mean (g)	0.51354	0.5112	0.595	0.46126	0.44214	0.44642	0.49782	0.37414
	S.D.	0.07977	0.0757	0.1628	0.12826	0.0284	0.11464	0.04608	0.09195
	N	5	5	5	5	5	5	5	5
Uterus	Mean (g)					0.56398	0.6236	0.69046	0.5483
	S.D.					0.29398	0.24662	0.27017	0.19195
	N					5	5	5	5

Discussion

The administration of the test material, SYN545192, to rats for a period of twenty-eight days at dietary concentrations of 100, 400 and 1200 ppm resulted in reduced body weight and body weight gain at 1200 ppm in both males and females.

Clinical chemistry investigations indicated that there was a reduction in plasma total protein and albumin levels in females at all doses, a slight increase in aspartate aminotransferase activity levels in 1200 ppm females and a reduction in plasma glucose levels was observed for the 1200 ppm males.

Increases in liver and heart weights were evident in males treated at 1200 ppm.
Microscopic examination of the tissues identified minimal tubular basophilia in the kidneys of females at 400 and 1200 ppm. The remaining histopathological changes were confined to the liver with centrilobular hepatocyte hypertrophy observed in males treated at 400 and 1200 ppm. There were no microscopic changes in the heart in males treated at 1200 ppm.

Investigators' conclusions – Oral (dietary) administration of SYN545192 to rats for a period of twenty-eight days resulted in a toxicologically significant reduction in body weight and body weight gain at 1200 ppm.
Minimal tubular basophilia was observed in the kidneys of females at 400 and 1200 ppm.
Minimal centrilobular hypertrophy was observed in males at 400 and 1200 ppm.

A clear "No Observed Effect Level" (NOEL) was established at 100 ppm, equivalent to 9 mg/kg/day in both males and females.

Reviewer comments – While the incidence of tubular basophilia is known that spontaneously occurs in rodents, in this study the incidence of tubular basophilia is not reflected in the control animals (0/5 females and 1/5 males).
Minimal tubular basophilia was observed in the kidneys of females at 100 (1/5), 400 (2/5) and 1200 ppm (4/5) and males at 100 (4/5), 400 (2/5) and 1200 ppm (0/5). Although the author mentions that Minimal tubular basophilia was observed in the kidneys of females at 400 and 1200 ppm and in males at 400 and 1200 ppm.

Considering the changes observed in the incidence of tubular basophilia in animals exposed to 100 ppm of SYN545192, the NOEL could be less than 100 ppm.

Study deficiencies – This study not presented certificate of analysis, deficiencies do not impact the assessment of the test substance.
In this study not reported the SYN545192 purity.

Reference

Peter CP, Burek JD, van Zwieten MJ (1986). Spontaneous nephropathies in rats. Toxicol Pathol 14(1):91-100.

B.6.3 Short-term toxicity

B.6.3.1 ORAL 28-DAY TOXICITY (RODENTS)

Study Type: SYN545192 - 28 Day Mouse Dietary Toxicity Study; Supplemental to OPPTS 870.3050

Test Material (purity): 98.3%

Citation: SYN545192 – 28 Day Mouse Dietary Toxicity Study. Charles River Tranent Edinburgh, EH33 2NE, UK. Laboratory report number: 30293. Study completion date: September 14, 2010.

Sponsor: Syngenta Crop Protection, LLC, USA.

Executive Summary: The purpose of the study was to assess the potential toxicity of the SYN545192 in the mouse after oral administration via the diet for 4 weeks.
Groups of 5 male and 5 female CD-1 mice were fed diets containing 0, 100, 300 or 500 ppm SYN545192 for a period of at least 28 days. Additional satellite groups of 7 control male and 7 female animals, and 15 male and 15 female animals at all other dose levels, were incorporated onto the study for toxicokinetic sampling and analysis.
The animals were monitored regularly for viability and for signs of ill health or reaction to treatment. Body weights and food consumption were measured and recorded at predetermined intervals from pretrial up until the completion of treatment. Blood samples were collected from all Main Study animals prior to necropsy for haematology and clinical chemistry analysis. Toxicokinetic blood samples were collected from designated Satellite Study animals over Days 25 and 26 of the study.

Animals treated at 500 ppm had initial body weight loss and statistically significantly lower mean body weight from Day 2 until the end of treatment. Following histopathological evaluation tubulointerstitial nephritis was observed in the kidneys of 2 males and 1 female treated at 500 ppm.

Males treated at 300 ppm had initial body weight loss and statistically significant lower body weights than controls between Days 2-5 of treatment. Although there was recovery towards control values from Day 7 onwards, mean body weight remained lower than controls throughout the study period.

Observed Effect Level (NOEL) was 100 ppm in both sexes, equating to 15.6 mg/kg/day in males, and 19.0 mg/kg/day in females.

Materials and Methods

Materials

Test material:	SYN545192	
Description:	White solid	
Lot/Batch #:	TE-6341	
Purity:	98.3%	
Test species:	Mice	
Strain:	CD-1 mice (Crl:CD-1(ICR))	
Sex	75 males and 75 females	
Age at start:	4 weeks old	
Weight at start:	10-14 g for males 11-13 g for females	
Source:	Charles River UK Limited,	
Housing:	The animals were housed singly (males) and 2 or 3 (females) per cage by sex and dose group in suspended polycarbonate cages (overall dimensions 48 x 15 x 13 cm) with stainless steel grid tops, integral food hoppers and solid bottoms.	
Diet:	Rat and Mouse (modified) No. 1 Diet SQC Expanded (Fine Ground) was supplied by Special Diets Services Limited, 1 Stepfield, Witham, Essex and available *ad libitum*.	
Water:	Filtered and sterilized well water, *ad libitum*	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	19-23°C 40-70% 15 air changes per hour. Light hours were 0700-1900 h.
Acclimation:	28 days prior to the administration of experimental diets.	

Study design and methods

Study experimentation dates - Start: November 14, 2008 End: March 16, 2010

Treatment

Dose groups

Animals were allocated to dose groups as in the table below:

Group	SYN545192 Treatment (ppm)		Animals			
			Study Main		Toxicokinetic Study	
			Males	Females	Males	Females
1	Control	0	1-5	21-22, 149-150, 25	41-47	93-99
2	Low	100	6-10	26-30	48-62	100-114
3	Intermediate	300	11-15	31-35	63-77	115-129
4	High	500	16-18, 146-147	36-40	87-92	130-144

During Pretrial, in order to standardise body weights across treatment groups, animals 19, 20, 23 and 24 were replaced by animals 146, 147, 149 and 150 respectively.

Route and duration of administration

Animals were dosed continuously via the diet for at least 28 days.
Control animals received blank diet (without test item under investigation) only.

Preparation of diet formulations
Diet formulations were prepared by dilution from a more concentrated stock at 5000 ppm. The 5000 ppm stock was prepared by firstly mixing the test item with the required amount of untreated control diet (Premix) in an automated mortar and pestle and mixed until visibly homogeneous. The premix was then blended with the required amount of untreated diet and blended for 20 min in a diet mixer (Winkworth). The 5000 ppm stock was then mixed with the required amount of untreated diet and blended for 20 min in a diet mixer (Winkworth) to prepare each of the treated dose groups.
Diet formulations were prepared fortnightly and stored at room temperature in the dark.
Formulations were dispensed once per week.
Blank diet (Rat and mouse (modified) No. 1 Diet SQC Expanded (Fine Ground)) was prepared for Control animals.

Toxicokinetic Analysis
Designated Satellite study animals were used for toxicokinetic blood sampling on Days 25/26 of treatment.

Sample analysis
Study samples were extracted and analysed in batches together with calibration standards and QC samples using the established method. The calibration samples were injected twice; at the beginning and end of the batch.
Concentrations of SYN545192 and its 5 metabolites (Desmethyl, Hydroxy, Desmethyl Hydroxy 1, Demethyl Hydroxy 2 and Desmethyl Hydroxy Dehydrated) in the plasma samples were determined at Charles River.
As no reference material was available for the metabolites, they were quantified against the parent standard line.

Clinical Pathology
Blood samples for hematology and clinical chemistry were obtained from all main study animals in random order via the orbital sinus under isoflurane anesthesia prior to terminal kill. The animals were not deprived of food overnight prior to sampling. Approximately 0.5 mL of whole blood was transferred into tubes containing EDTA for hematology investigations, and assayed for the following parameters:
For clinical chemistry approximately 0.5 mL was transferred into plastic tubes containing lithium heparin, which were then centrifuged and assayed for the following parameters:

Hematology Parameters	Clinical Chemistry Parameters
Haemoglobin	Alkaline Phosphatase
Red Blood Cell Count	Alanine Aminotransferase
Haematocrit	Aspartate Aminotransferase
White Blood Cell Count	Creatinine
Mean Cell Volume	Urea
Mean Cell Haemoglobin	Glucose
Mean Cell Haemoglobin Concentration	Cholesterol
Platelets	Triglycerides
Reticulocytes	Albumin
Differential White Blood Cell Count: Neutrophils Lymphocytes Monocytes	Total Protein
	Globulin – derived
	AG Ratio – derived
	Phosphate

Eosinophils Eosinophils Large Unclassified Cells	Calcium
	Creatine Kinase
	Gamma Glutamyl Transferase
	Total Bilirubin
	Sodium
	Potassium
	Chloride

Terminal Studies

After 28 days of treatment all Main Study animals were killed in a random order by exposure to carbon dioxide and had their terminal body weight recorded followed by exsanguination.

Each animal was subjected to a detailed necropsy under the guidance of a veterinary pathologist, who visited the post-mortem room on the day of necropsy and remained on call throughout the necropsy session. The necropsy consisted of a complete external and internal examination, which included body orifices (ears, nostrils, mouth, anus, and vulva) and cranial, thoracic and abdominal cavities. All gross findings were recorded in terms of location(s), size (in mm), shape, colour, consistency and number.

Electronic Data Evaluation Systems

The following Data capture software was used (details pertaining to the software versions used are retained in the study data files):

- In-life data collection and reporting: Provantis
- Necropsy and histopathology data collection and reporting: Places 2000
- Test item control and preparation: Dispense
- Chromatography: Atlas 2002
- Clinical Pathology Laboratory Information Management System (LIMS): Nautilus 2003
- Bioanalysis of Toxicokinetic samples: Watson

Data Evaluation

Body weight gain, body weight and food consumption data were analysed using a parametric ANOVA and pairwise comparisons made using the Dunnets t-test distribution. If the variances were heterogeneous, log or square root transformations were used in an attempt to stabilize the variances.

Organ weights were analyzed as above and by analysis of covariance (ANCOVA) using terminal kill body weight as covariate.

Analyses of variance and covariance were carried out using the MIXED procedure in SAS (9.1.3). Least-squares means for each group were calculated using the LSMEAN option in SAS PROC MIXED. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two sided Dunnet's t-test, based on the error mean square in the analysis.

Summary statistics (mean, standard deviation and number of observations) and individual values are presented for organ weights as a percentage of body weight. However, statistical comparisons to control values were not performed for relative organ weight (%), because the ANCOVA results with terminal body weight as covariate provide a more robust statistical determination of this parameter.

The following pairwise comparisons were performed:

- Control vs Low Dose
- Control vs Intermediate Dose
- Control vs High Dose

Histopathology data were analyzed using Fishers Exact Probability Test.
All statistical tests were two-sided and performed at the 5% significance level using in-house software. Males and females were analyzed separately.

Results

Achieved Dosage

The overall mean achieved dose levels were 0, 15.6, 47.4 and 81.8 mg SYN545192/kg/day for males and 0, 19.0, 57.9 and 91.5 mg/kg/day for females, corresponding to dietary inclusion levels of 0, 100, 300 and 500 ppm respectively.

Observations

Mortality

There were no premature decedents during the study.

Incidence of clinical observations

Male animal 18, treated at 500 ppm was noted with weight loss, hunched body and incidences of piloerection between Days 10-18.

Body weight and weight gain – Body weight was statistically significantly lower in both males and females treated at 500 ppm from Day 2 until the end of treatment. This was accompanied by statistically significantly lower body weight gain at alltime points.
In males treated at 300 ppm, body weight was noted to be statistically significantly lower compared to their controls from Day 2 to Day 5, accompanied by a statistically significantly lower body weight gain at Day 0-3. From Day 6 onwards body weight and body weight gain showed some signs of recovery, however throughout treatment it was noted to be lower compared to their control group.
There were no notable differences in bodyweight in either sex treated at 100 ppm compared to their respective controls.

Table 1. Average body weights (g) during 28 days of treatment

Group:		1	2	3	4										
Test Item:		Control	SYN545192	SYN545192	SYN545192										
Dosage (ppm):		0	100	300	500										
Group / Sex		Day													
		0	1	2	3	4	5	6	7	10	14	17	21	24	28
1 M	Mean	39	39	39.3	39.1	39.2	39.1	38.6	38.2	39.7	40.3	40.1	41.2	41.5	42.3
	SD	2	1.8	2.1	1.7	1.6	2.3	2.2	2.2	1.7	1.3	2.7	1.7	2.2	2.2
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2 M	Mean	37	37	37.4	37	36.8	36.7	37.1	36.8	37.3	37.5	38.1	39	39.6	39.4
	SD	2.1	2	1.9	2.1	2.1	2.1	2.2	1.9	1.7	1.2	1.4	2	2.1	1.8
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3 M	Mean	36.7	36.3	35.8*	35**	35.6**	35.9*	36	35.7	36	37.4	37.8	38.2	38.8	39.5
	SD	2.1	1.9	1.4	1.5	1.6	1.8	1.7	2.2	2.4	2.1	2.3	2.3	2.3	2.5
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4 M	Mean	37.2	36.1	34.3**	32.9**	32.6**	32.5**	32.6**	31.3**	30.5**	31.9**	32.8**	33.3**	33.5**	33.4**
	SD	1.7	1.6	1.2	0.9	0.9	1.1	1.8	2.4	3.2	3.2	3.5	3.8	4.4	4.5
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1 F	Mean	26.2	27	27.1	26.9	26.7	26.7	27	26.9	28	28.2	29.1	29.8	29.2	29.8
	SD	0.9	0.5	0.9	1.2	1.2	1.3	1	1.1	1.2	2.1	1.8	2	2	1.9
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2F	Mean	26.2	26.1	26.1	26	26	27	26.8	26.9	27.2	27.4	28.8	28.5	28.6	28.7
	SD	0.5	0.9	1	0.9	0.7	0.8	1	0.5	0.7	1.2	1.9	1.7	0.7	1.4
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3F	Mean	25.6	25.9	25.2	24.9	25.6	26.3	26.4	26	27	27.9	28.1	28.8	28.9	28.7
	SD	2.2	2.5	2.2	2.2	2.1	2	1.9	2	2.4	2.9	2	1.9	2.2	1.6
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4F	Mean	25.9	24.6	23.2**	22.0**	23.5*	23.3*	23.7*	22.4**	23.1**	24.9	24.7**	25.3*	25.7*	26.6*
	SD	1.8	1.9	1.6	1.5	2.5	3	3.1	2.9	2.7	2.5	2.2	2.8	2.3	1.2
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Significantly different from Group 1: *=p<0.05, **=p<0.01

Food consumption - Isolated statistically significant decreases in food consumption were noted at Day 5 in male animals treated at 100 and 500 ppm, and at Days 3 and 24 in females treated at 500 ppm compared to their respective controls. Due to the isolated nature of the differences, and the lack of any correlation with the changes in body weights, these were considered not to be related to treatment.

Toxicokinetics – This study describes the determination of concentrations of SYN545192 in mouse plasma. Toxicokinetic evaluation showed that the mice were continuously exposed to measurable concentrations of SYN545192 at all dose levels. In control animals, levels of SYN545192, and of its 5 metabolites, were below the limit of quantification at all time points.

Table 2. Quality Control Samples Results for SYN545192 (Plasma)
Results expressed as ng/mL

Batch	Low	MID	HIGH
	10.0	80.0	800
1	9.45	78.2	726
	11.3	67.4	722
	8.77	81.1	666
	10.6	79.3	855
	9.99	69.2	727
	10.8	74.7	738
2	9.79	77.4	795
	7.07*	82.8	759
	9.14	82.1	741
Mean	9.66	76.9	748
S.D.	1.27	5.50	52.7
CV (%)	13.1	7.2	7.0
Accuracy (%)	96.6	96.1	93.5
Bias (%)	-3.4	-3.9	-6.5
n	9	9	9

* = Outside acceptance criteria (±20%), included in statistical calculations

Blood analyses

Haematology – Statistically significant higher values were noted in the red blood cell counts in males treated at 500 ppm, and in large unclassified cells in females at 500 ppm. However, due to no related histological findings and the fact that the values were within historical control ranges (9.28 - 9.52 x 10^{12}/L) this change was considered not to be treatment related.

At 100 ppm haemoglobin, and consequently mean cell haemoglobin concentration, were both statistically significantly increased in males, and the red blood cell count was statistically significantly increased in females. However, due to the lack of any differences at 300 and 500 ppm and therefore a dose related response, the small magnitude of the difference, and values were within (haemoglobin) or very close (red blood cell count, mean cell haemoglobin concentration) to the historical control ranges, these were considered not to be related to treatment.

Table 3. Group Mean Hematology

Group:	1	2	3	4
Test Item:	Control	SYN545192	SYN545192	SYN545192
Dosage (ppm):	0	100	300	500

Group / Sex		Hb g/dL	RBC $x10^{12}$/L	Hct L/L	MCH pg	MCV fL	MCHC g/dL	Ret %	WBC	Neut	Lymp	Mono	Eos	Baso	LUC	Plat
												$x10^9$/L				$x10^9$/L
1 M	Mean	13.2	8.32	0.413	15.9	49.7	31.9	3	4.81	0.63	3.9	0.13	0.12	0	0.03	1274
	SD	0.7	0.42	0.021	0.4	1.5	0.5	0.5	2.28	0.26	1.9	0.06	0.07	0.01	0.02	215
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2 M	Mean	14.3*	8.96	0.437	16	48.7	32.8*	3.1	4.74	0.65	3.8	0.13	0.14	0	0.03	1254
	SD	0.9	0.46	0.029	0.4	1.1	0.3	0.3	1.21	0.31	1.05	0.07	0.08	0.01	0.01	132
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3 M	Mean	13.6	8.58	0.425	15.8	49.5	32	3	5.48	0.87	4.25	0.18	0.13	0.01	0.04	1380
	SD	0.4	0.36	0.012	0.2	1	0.6	0.2	2.67	0.5	2.06	0.1	0.03	0.01	0.05	80
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4 M	Mean	14.1	9.02*	0.432	15.6	48	32.6	3.1	5.5	1.29	3.94	0.12	0.11	0	0.03	1304
	SD	0.4	0.42	0.012	0.3	1	0.3	0.3	2.87	0.76	2.22	0.07	0.06	0	0.03	55
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1 F	Mean	13.4	8.29	0.407	16.2	49	33	2.9	6.04	0.68	4.93	0.12	0.26	0	0.04	1202
	SD	0.8	0.34	0.02	0.4	0.9	0.3	0.9	1.21	0.16	0.94	0.04	0.09	0.01	0.01	84
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2 F	Mean	14.2	8.95*	0.434	15.8	48.5	32.6	3	6.2	0.8	5.07	0.09	0.18	0.01	0.05	1154
	SD	0.6	0.15	0.01	0.5	1.2	1	0.8	2.04	0.3	1.66	0.04	0.08	0.01	0.02	143
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3 F	Mean	13.8	8.37	0.422	16.5	50.3	32.9	2.3	4	0.36	3.44	0.06	0.12	0	0.02	1369
	SD	0.8	0.18	0.026	1	2.1	2	0.6	0.8	0.13	0.73	0.02	0.11	0	0.01	273
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4 F	Mean	13.8	8.85	0.437	15.6	49.5	31.5	4.3	8.32	1.18	6.78	0.14	0.15	0.01	0.06*	1237
	SD	0.8	0.58	0.016	0.6	1.8	0.9	1.7	3.29	0.53	2.64	0.04	0.09	0.01	0.02	215
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Significantly different from Group 1: *=p<0.05, **=p<0.01

Clinical chemistry – In females treated at 500 ppm, chloride was statistically significantly higher and albumin was statistically significantly lower compared to their control. However, as the change was small in magnitude, was within the historical control range and was confined to one sex it was considered not to be related to treatment.

In females treated at 300 ppm, aspartate aminotransferase was noted to be statistically significantly lower than controls. At 100 ppm, both total bilirubin and creatinine were statistically significantly higher when compared to their control. These changes were only observed at the mid and low dose levels and were not seen in animals treated at 500 ppm.

Due to the lack of a dose response and the fact that the values were within (chloride, total bilirubin and creatinine) or below (aspartate aminotransferase, albumin) the historical control ranges, these findings were considered not to be

related to treatment with SYN545192.

Table 4. Group Mean Blood Chemical

Group:	1	2	3	4
Test Item:	Control	SYN545192	SYN545192	SYN545192
Dosage (ppm):	0	100	300	500

Group (Sex)		ALP iu/L	ALT iu/L	AST iu/L	GGT iu/L	CPK iu/L	Urea mmol/L	Glu mmol/L	T.Bil µmol/L	Chol mmol/L	Trig mmol/L	TP g/L	Alb g/L	Glob g/L	K mmol/L	Cl mmol/L	Phos mmol/L	Ca mmol/L	Crea µmol/L
1 (F)	Mean	89	48	75	3	161	7.4	11.03	1.6	1.9	1.15	50	34	16	4.1	115	2.26	2.37	6
	S.D.	26	27	22	0	124	0.8	1.68	0.2	0.6	0.58	2	1	2	0.2	1	0.22	0.12	2
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2 (F)	Mean	92	37	72	3	169	7.4	11.70	2.0*	2.5	0.95	51	34	17	3.8	114	2.36	2.32	12*
	S.D.	32	24	18	0	37	2.0	3.17	0.1	0.7	0.42	2	1	2	0.3	3	0.29	0.05	3
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3 (F)	Mean	67	24	51*	3	86	8.3	9.35	1.4	2.0	1.11	49	32	17	3.7	117	2.54	2.31	9
	S.D.	15	4	6	0	41	1.5	1.22	0.3	0.5	0.50	3	2	1	0.1	1	0.26	0.05	5
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4 (F)	Mean	86	22	54	3	191	8.8	9.63	1.7	2.0	0.53	49	31*	18	4.4	118*	2.59	2.35	11
	S.D.	31	6	4	0	77	0.9	1.78	0.2	0.1	0.15	3	0	2	0.3	1	0.31	0.04	1
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
1 (M)	Mean	88	47	55	3	177	7.4	11.51	2.2	3.5	1.19	52	32	20	4.4	114	2.33	2.36	9
	S.D.	36	15	19	0	213	1.1	0.88	0.6	0.7	0.58	3	1	2	0.4	2	0.37	0.08	4
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2 (M)	Mean	70	37	46	3	94	7.6	13.81	2.9	3.3	1.14	50	30	20	4.9	115	2.38	2.33	9
	S.D.	25	17	8	0	46	1.7	2.08	1.0	0.8	0.36	2	1	1	0.4	4	0.33	0.07	3
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3 (M)	Mean	70	41	50	3	79	7.6	11.38	2.3	3.2	1.31	54	32	22	4.1	113	2.13	2.40	10
	S.D.	18	15	7	0	54	1.3	1.54	0.5	0.7	0.56	2	1	1	0.4	3	0.23	0.05	3
	N	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.0	5.00	5.00	5.00	5.00	5.00	5.00	5.00
4 (M)	Mean	60	37	58	3	219	13.0	9.32	1.7	3.9	1.05	55	32	23	5.0	114	2.32	2.44	13
	S.D.	11	8	11	0	236	8.8	2.53	0.6	1.6	0.49	6	3	4	0.7	2	0.48	0.13	5
	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Significantly different from Group 1: *=p<0.05, **p<0.01

Organ weights - Absolute heart and kidney weights were both statistically significantly lower in males treated at 500 ppm compared to their control. However, as terminal body weight was also statistically significantly lower, and there were no corresponding changes seen in the relative or adjusted organ weights, this difference was considered to be a consequence of the reduction in body weight, and not a direct effect to treatment with SYN545192.
Covariate brain weight was also noted to be statistically significantly higher in males treated at 500 ppm compared to their control; however, there were no differences in absolute organ weight.

Histological findings - Dietary administration of SYN545192 was associated with a small increase in the incidence of tubulointerstitial nephritis in the kidneys of both sexes at 500 ppm when compared to controls:

Males Females
Group 1: 0/5 0/5
Group 3: 0/5 0/5
Group 4: 2/5 (minimal) 1/5 (slight)

The tubulointerstitial nephritis was characterized by tubular basophilia accompanied by an interstitial inflammatory cell infiltration. Minimal tubular dilatation was a component of this finding in both affected male animals but was not present in the single affected female. All other recorded histological findings were typical of the spontaneously arising background alterations observed in mice of this strain and age.

Discussion

Investigators' conclusions – Daily administration of SYN545192 at a dose of 500 ppm for at least 28 consecutive days produced a clear reduction in body weight in both males and females and was also associated with a small increase in the incidence of tubule interstitial nephritis in the kidneys of both sexes. At 300 ppm, there was also initial body weight loss and statistically significantly lower body weight compared to their controls from Day 2 to Day 5.
There were no adverse treatment related effects in males or females treated at 100 ppm. In conclusion, based on the findings seen from this study, it was considered that a clear No Observed Effect Level (NOEL) was 100 ppm in both sexes, equating to 15.6 mg/kg/day in males, and 19.0 mg/kg/day in females.

Reviewer comments – The results of blood chemistry different changes, principally females, are observed in the three concentrations (100, 300 y 500 ppm). At 100 ppm, both total bilirubin and creatinine were statistically significantly higher when compared to their control. At 300 ppm, aspartate aminotransferase was noted to be statistically significantly lower than controls. At 500 ppm, chloride was statistically significantly higher and albumin was statistically significantly lower compared to their control. While it is true, the change was small in magnitude. In relation to this, it is interesting to note that the values de ALT, AST, GLU, TRIG in the group of males decreasing trend is observed doses related compared to their respective controls, however are not statistically different.

While it has been a decrease in food intake in animals treated with SYN545192 to 100 and 500 ppm. and no relationship is observed in the weight of animals at the same concentrations, the authors mention that these changes are not related to treatment. However, we believe that the weight loss in the animals treated with 500 ppm of SYN545192 itself could be related to exposure to SYN545192 because the weight loss of the animals is maintained from day 2 until the end of treatment in both males and females.

In histological results where summarizes the incidence of tubular basophilia apparently has a mistake, because at the histological results from mice exposed to 500 ppm of SYN545192 Appendix 13 of the original study (page 164) shows that males present tubular basophilia (2/5) and in females present slight tubular basophilia (2/5) and minimal (1/5) slight.

While the incidence of tubular basophilia is known that spontaneously occurs in rodents, in this study the incidence of tubular basophilia is not reflected in the control animals (2/5 females and 0/5 males) or in the concentrations of 100 and 300 ppm, but the impact is induced in the animals treated at the concentration of 500 ppm. Therefore we believe that the incidence of tubular basophilia could be due to the processing of 500 ppm SYN545192. In the literature mentioned that the severity and incidence of the spontaneously occurring tubular basophilia can also be altered by various chemical agents (Peter CP., et al., 1986).

Considering the changes observed in the bilirubin, creatinine, and the incidence of tubular basophilia in animals exposed to 100 ppm of SYN545192, the NOEL could be less than 100 ppm.

Study deficiencies – None

Reference

Peter CP, Burek JD, van Zwieten MJ (1986). Spontaneous nephropathies in rats. Toxicol Pathol 14(1):91-100.

Short-term toxicity

Study Type: SYN545192 – 90 Day Dietary Study in Rats; OPPTS 870.3100 (rodent); OECD 408; EU Directive 96/54/EC B.26.

Test Material (purity): 98.3%

Citation: SYN545192 – 90 Day Dietary Study in Rats. Charles River, Tranent Edinburgh, EH33 2NE UK. Laboratory report number: 30138. Study completion date: September 13, 2010.

Sponsor: Syngenta Crop Protection, LLC, USA.

Executive Summary: In a subchronic toxicity study, SYN545192 was administered to Han Wistar 10 rats/sex/dose in the diet at dose levels of 0, 100, 750 or 1500 ppm for 90 days.

Daily administration of SYN545192 for at least 90 consecutive days at 750 ppm and above resulted in a significant reduction in body weight, food consumption and food utilisation.

Statistically significant decreases were noted in alkaline phosphatase and glucose in both sexes at 1500 ppm, and in females only at 750 ppm. Additionally, there was a statistically significant increase in urea in males at 750 or 1500 ppm.

Centrilobular hypertrophy of the liver was seen in males at 750 ppm above and in females treated at 1500 ppm.

In conclusion, under the conditions of the study, a clear No Observed Effect Level (NOEL) was considered to be 100 ppm for both males and females equating to an average of 7.6 mg/kg/day in males and 8.2 mg/kg/day in females.

This subchronic toxicity study in the rat is acceptable and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3100; OECD 408) in rat.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Materials and Methods

Materials

Test material:	SYN545192
Description:	white powder
Lot/Batch #:	TE-6341
Purity:	98.3%
Vehicle:	Not reported
Positive control:	Not reported
Test species:	Rats

Strain:	Han Wistar rats (CRL: WI(Han))	
Sex:	42 male and 42 female	
Age at start:	7 weeks old	
Weight at start:	186 - 244 g for males 145 - 187 g for females	
Source:	Harlan Laboratories UK Ltd	
Housing:	The animals were housed 2 per cage by sex and dose group in suspended polycarbonate cages (overall dimensions 61 x 43.5 x 24 cm) with stainless steel grid tops, integral food hoppers and solid bottoms.	
Diet:	Rat and Mouse (modified) No. 1 Diet SQC Expanded (Fine Ground) was supplied by Special Diets Services Limited, 1 Stepfield, Witham, Essex and available *ad libitum.*	
Water:	Animals had access to domestic mains water ad libitum except during motor activity assessment and urine collection	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	19-23°C 40-70% 15 air changes per hour Light hours were 0700-1900 hour
Acclimation:	2 weeks prior to the administration of experimental diets.	

Study design and methods

Study experimentation dates: Start: End:

Animal assignment

Table 1. Dose groups
Animals were allocated to dose groups as in the table below:

Group		SYN545192 Treatment (ppm)	Number of Animals	
			Male	Female
1	Control	0	10	10
2	Low	100	10	10
3	Intermediate	750	10	10
4	High	1500	10	10

Diet preparation and analysis – A dietary stock premix at 5000 ppm was prepared by firstly making 200g of premix containing the total amount of test item required to produce the final concentration of diet requested of each dose group.
Diet at a concentration of 1500 ppm was prepared by accurately weighing the required amount of stock formulation and adding to an appropriate amount of blank RM1 diet.
Diet formulations in the range of 100-5000 ppm were prepared twice weekly during Weeks 1 and 2, thereafter were prepared weekly and stored at ambient temperature in the dark prior to administration of the animals.
Blank diet (Rat and Mouse (modified) No.1 Diet SQC Expanded (Fine Ground)) was prepared for the control animals.
Stability data for diets at 10-5000 ppm was confirmed for 30 days when stored at ambient temperature in the dark, following work carried out in the Analytical Chemistry Services of Charles River, Edinburgh under a separate protocol (Charles River Study No. 425169, Method No. 2516).

Statistics – Body weight, cumulative body weight gain, food consumption, haematology, coagulation, clinical chemistry, selected urinalysis parameters, motor activity and quantitative FOB measurement data were analyzed using

a parametric ANOVA and pairwise comparisons made using the Dunnett's t-test distribution. If the variances were heterogeneous, log or square root transformations were used in an attempt to stabilize the variances.

Organ weights were analyzed as above and by analysis of covariance (ANCOVA) using terminal kill body weight as covariate.

Summary statistics (mean, standard deviation and number of observations) and individual values are presented for organ weights as a percentage of body weight. However, statistical comparisons to control values were not performed for relative organ weight (%), because the ANCOVA results with terminal body weight as covariate provide a more robust statistical determination of this parameter.

The following pairwise comparisons were performed:
Control vs Low Dose
Control vs Intermediate Dose
Control vs High Dose
Histopathology data were analysed using Fishers Exact Probability Test.
Functional Observation battery parameters that yielded descriptive data were analysed by Fisher's Exact Test.
All statistical tests were two-sided and performed at the 5% and 1% significance level using in-house software. Males and females were analyzed separately.

Methods

Observations – All animals were checked early morning and as late as possible each day for viability. Once each week, all animals received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.

Functional observations – Once during the treatment period (Week 12/13), the following additional functional tests were performed. Again, these assessments were performed at an approximately standardized time of day. The functional tests are: Grip strength, Pain perception, Landing Foot Splay, Motor activity.

Body weight – Body weights were recorded twice, daily during the first week and then twice weekly thereafter until the completion of treatment.

Food consumption and utilisation– The quantity of food consumed by each cage of animals was measured and recorded twice prior to commencing treatment, daily during the first week then twice weekly thereafter until the completion of treatment.

Food utilisation was calculated for Weeks 1-4, 5-8, 9-13 and 1-13 according to the following formula:
(Cage mean weight gain x 100) / cage total food consumption
Reported values show the amount of weight gained (g) for every 100g of food consumed

Ophthalmoscopic examination - The cornea, anterior chamber, iris, lens, posterior chamber, retina and vessels of the optic disc were examined. An ophthalmic examination was conducted for all animals during Pretrial and Week 13. The eyes were examined using an indirect ophthalmoscope following application of a mydriatic agent (1% Tropicamide, Mydriacyl®).

Hematology and clinical chemistry - Blood samples for haematology, coagulation and clinical chemistry were obtained from all animals *via* the orbital sinus under isoflurane anaesthesia prior to necropsy. The animals were not deprived of food overnight prior to sampling.

Approximately 0.45 mL blood was transferred into tubes containing 0.05 mL trisodium citrate (w/v) for assessment of coagulation.

Approximately 1.0 mL was taken into lithium heparin tubes, to be used for clinical chemistry investigations.

The checked (X) parameters were examined.

Hematology Parameters

X	Hematocrit (Hct)		**DIFFERENTIAL WHITE BLOOD CELL COUNT:**
X	Hemoglobin (Hb)	X	Neutrophils (Neut)
X	White Blood Cell Count (WBC)	X	Lymphocytes (Lymph)
X	Red Blood Cell Count (RBC)	X	Monocytes (Mono)
X	Platelet (Plat)	X	Eosinophils (Eos)
X	Mean Cell Volume (MCV)	X	Basophils (Baso)
X	Mean Cell Haemoglobin (MCH)	X	Large Unclassified Cells (LUC)
X	Mean Cell Haemoglobin Concentration (MCHC)		
X	Reticulocytes (Ret)		

Coagulation Parameters

X	Prothrombin Time (PT)
X	Activated Partial Thromboplastin Time (APTT)

Clinical chemistry Parameters

X	Urea (Urea)	X	Total Protein (TP)
X	Glucose (Glu)	X	Albumin (Alb)
X	Aspartate Aminotransferase (AST)	X	Globulin (Glob)
X	Alanine Aminotransferase (ALT)	X	AG Ration (AG-R)
X	Alkaline Phosphatase (ALP)	X	Cholesterol (Chol)
X	Gamma Glutamyl Transpeptidase (GGT)	X	Triglycerides (Trig)
X	Glutamate Dehydrogenase (GLDH)	X	Creatinine (Crea)
X	Creatine kinase (CPK)	X	Total Bilirubin (T.Bil)
X	Sodium (Na)	X	Calcium (Ca)
X	Potassium (K)	X	Phosphate (Phos)
X	Chloride (Cl)		

The limit of detection for the following assays was observed and reported as follows:

Assay	Limit of Detection	Non-detectable values reported as
Gamma Glutamyl Transpeptidase	3 iu/L	<3 iu/L
Total Bilirubin	1.7 µmol/L	<1.7 µmol/L
Glutamate Dehydrogenase	1 iu/L	<1 iu/L

For the purposes of calculating mean values the lower limit of detection has been used for all non-detectable parameters.

Urine samples were collected over a 4 h period from all animals during Week 11. The animals were housed individually in metabolism cages and were deprived of food and water.

Urinalysis Parameters

X	Volume (Uvol)	X	Urobilinogen (Uro)
X	Specific Gravity (S.G.)	X	Urinary Bilirubin (U.Bi)
X	Urine Colour (Ucol)	X	Blood Pigments (BP)
X	pH (UpH))	X	**MICROSCOPY OF THE SPUN DEPOSIT:**
X	Urinary Protein (UProt)		Epithelial Cells (Mic.E), Crystals (Mic.Cr), White Blood Cells (Mic.W), Red Blood Cells (Mic.R), Organisms (Mic.O), Casts (Mic.C), Other Abnormalities (Mic.A)
X	Urinary Glucose (UGl)		
X	Urinary Ketones (UKet)		

Terminal Studies

After at least 90 days of treatment all animals were killed in a randomised order by exposure to carbon dioxide and had their terminal body weight recorded followed by exsanguination.

Each animal was subject to a detailed necropsy under the guidance of a veterinary pathologist, who visited on the first day of necropsy and remained on call throughout the necropsy period. The necropsy consisted of a complete external and internal examination, which included body orifices (ears, nostrils, mouth, anus and vulva) and cranial, thoracic and abdominal cavities. All gross findings were recorded in terms of location(s), size (mm), shape, colour and number.

The organs marked ‘x’ in the ‘Weighed’ column in the table below were removed and weighed before preservation. Paired organs were weighed separately and the sum of the individual organs used for reporting purposes.

Representative samples of tissues listed in the table below were taken from all animals and fixed in 10% neutral buffered formalin, unless otherwise stated.

Tissues marked ‘x’ in the ‘Examined’ column, in the table below, were processed to block from all animals. Sections were cut 4-6 µm thick and stained with haematoxylin and eosin (H&E) (unless otherwise stated) from all animals in the Control and High dose group and evaluated by a pathologist. In addition the liver was also processed and examined from all animals in the Low and Intermediate groups. Duplicate femoral bone marrow smears were also taken at necropsy and one was stained using May-Grunwald-Giemsa Romanowsky stains for possible examination. This smear was evaluated, with the second, unstained, smear being retained in the pathology department.

Tissues collected	Weighed	Examined	Comments
Abnormal Tissue	-	x	-
Adrenal x 2	x	x	-
Aortic Arch	-	x	-
Blood sampling	-	-	via the vena cava
Blood smear	-	-	From animals killed prematurely
Brain	x	x	Forebrain, midbrain, cerebellum and pons.
Cervical Lymph Nodes	-	x	-
Diaphragm	-	x	-
Epididymis x 2	x	x	-
Eye x 2 .	-	x	Both eyes were fixed in Davidson's fluid. One only was processed and examined

Gastro-intestinal Tract: Stomach Duodenum Jejunum Ileum Caecum Colon Rectum	 - - - - - - -	 x x x x x x x	Opened at necropsy and mucosa examined. Peyers patches were sampled from small intestine.
Harderian Gland .	-	x	Both fixed in Davidson's fluid. Only one processed and examined
Heart	x	x	-
Implant	-	-	For identification purposes.
Kidney x 2	x	x	Transverse section taken from both kidneys and processed to paraffin wax block after 48-72h. A small section of duodenum was incorporated into each block.
Larynx	-	x	-
Liver	x	x	Three lobes fixed with 2 lobes examined. Representative sections of left lateral lobe, right median lobe and caudate lobe were taken and processed to paraffin wax block after 48-72h. A small section of duodenum was incorporated into each block.
Lung	-	x	All lobes were examined, including mainstream bronchi.
Marrow Smear (femur) .	-	x	Bone marrow smears were taken from all animals, air dried and fixed in methanol.
Mesenteric Lymph Node	-	x	-
Nasal Cavity	-	x	Transverse section examined
Oesophagus	-	x	-
Optic Nerve x 2	-	x	Fix in Davidson's fluid. Only one examined.
Ovary x 2	x	x	-
Oviducts	-	x	-
Pancreas	-	x	-
Parotid Salivary Gland	-	x	Only one processed and examined.
Pharynx	-	x	-
Pituitary	-	x	-
Prostate	-	x	-
Rib	-	-	Including costochondral junction.
Sciatic Nerve	-	x	-
Seminal Vesicles	-	x	-

Skin + Mammary Gland	-	x	-
Soleus Muscle	-	x	-
Spinal Cord	-	x	Cervical, midthoracic and lumbar regions.
Spleen	x	x	-
Sternum	-	x	Including bone marrow
Submandibular Lymph Node	-	x	-
Submaxillary (Mandibular) Salivary Gland (including sublingual)	-	x	Only one processed.
Testis	x	x	Weighed individually, then fixed in Modified Davidson's fluid, trimmed after ca 18-24 h then transferred to Neutral Buffered Formalin for a further 3-4 h. Trimmed tissue then transferred to alcohol, prior to processing for examination.
Thigh Muscle	-	x	-
Thymus	x	x	-
Thyroid with Parathyroid x 2	x	x	Weighed together afte fixation. Sectioned into its 2 constituent lobes; one thyroid processed to paraffin wax block after 48-72h. A small section of duodenum was incorporated into each block.
Tongue	-	x	-
Trachea	-	x	-
Urinary Bladder .	-	x	Contracted bladders distended with fixative; epithelial surface in animals which underwent histological evaluation were examined after fixation
Uterus+Cervix	x	x	Sectioned longitudinally and processed to paraffin wax block after 48-72h. A small section of duodenum was incorporated into each block.
Vagina	-	x	-

Results

Mortality There was one premature decedent on this study. A rat male was found dead at 78 day of SYN545192 treatment 1500 ppm. Lungs reddened, Mandibular lymph node reddened were the major necropsy findings.
The premature death was considered to be procedure related (toxicokinetic blood sampling) and not associated with treatment with SYN545192.

Incidence of clinical observations - There were no clinical signs which could be positively attributed to consumption of diets containing SYN545192.

Body weights and cumulative body weight gain - Group mean body weights were statistically significantly lower than controls in animals treated at 750 or 1500 ppm. At 1500 ppm, mean body weights were statistically significantly lower from Day 31 in males and Day 3 in females. At 750 ppm, group mean body weights were statistically significantly lower than controls from Day 38 in males and Day 14 in females. Correspondingly, body weight gain was noted to be significantly lower in all animals treated at 750 and 1500 ppm at all timepoints compared to their respective controls throughout the treatment period.

There were no changes noted in body weights for animals treated at 100 ppm compared to their respective control group.

Table 1. Body weights (g) during 90 days of treatment

Group:		1	2	3	4
Test Item:		Control	SYN545192	SYN545192	SYN545192
Dosage (ppm):		0	100	750	1500
Group / Sex		Day			
		0	7	14	21	28	31	38	49	56	63	70	77	84	91
1 M	Mean	214.8	250.7	278.4	302.4	323.9	333.9	352.3	380.2	392.6	405.0	413.4	423.4	429.9	436.5
	SD	15.0	19.1	24.3	26.4	28.7	28.9	31.2	34.4	34.8	35.9	36.0	37.0	38.8	42.9
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
2 M	Mean	219.9	254.8	279.5	303.7	322.7	328.9	346.6	370.8	382.9	391.4	399.9	411.2	417.7	423.1
	SD	11.5	16.6	20.6	23.4	27.0	27.6	29.5	34.6	34.9	32.3	36.0	36.5	36.1	35.0
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
3 M	Mean	212.7	239.3	261.1	283.7	300.3	308.3	320.8*	343.7*	355.4*	361.9*	370.2*	379.6*	383.4*	389.9*
	SD	12.6	15.0	19.9	23.6	25.6	26.5	26.4	27.1	27.7	27.5	27.6	27.3	30.3	30.7
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
4 M	Mean	174.6	237.0	257.8	281.3	298.1	302.4*	316.2*	332.4**	346.8**	350.7**	360.6**	368.7**	367.8**	373.8**
	SD	11.8	17.2	16.9	19.9	20.7	24.1	24.7	29.4	29.5	31.1	33.0	33.4	33.8	34.9
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10

		Days													
		0	3	14	21	28	31	38	49	56	63	70	77	84	91
1 F	Mean	166.9	177.3	198.8	212.3	223.4	226.8	230.0	238.8	242.5	244.9	249.2	252.8	256.6	257.0
	SD	12.1	14.2	16.0	17.4	19.7	19.9	19.2	19.4	19.0	20.5	20.0	19.2	18.8	20.5
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
2F	Mean	166.2	176.2	199.9	212.5	222.5	227.3	233.4	240.4	248.0	249.3	253.2	256.8	257.8	259.8
	SD	9.6	11.6	13.7	18.0	16.0	18.4	17.5	17.3	17.9	16.8	16.8	17.3	16.6	17.5
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
3F	Mean	164.7	167.8	184.5*	193.5*	200.3**	201.4**	205.7**	210.0**	215.6**	215.4**	218.3**	222.0**	221.9**	224.9**
	SD	9.7	12.3	10.7	10.7	13.1	13.9	11.3	11.2	13.1	13.6	12.9	13.7	13.3	14.0
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
4F	Mean	165.1	161.3*	168.3**	177.7**	184.5**	184.9**	190.3**	193.5**	199.9**	202.7**	202.4**	204.7**	203.7**	205.9**
	SD	7.7	7.2	6.9	7.6	8.9	7.6	9.0	7.3	9.0	8.7	8.3	10.9	11.4	9.2
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Significantly different from Group 1: *=p<0.05, **=p<0.01

Food consumption and food utilization - Food consumption was occasionally statistically lower through the treatment period in all animals treated at 750 or 1500 ppm. Overall food utilization was significantly lower in all treated groups with the exception of females treated at 100 ppm. Monthly utilization indicated a statistical reduction at all timepoints in males and females treated at 1500 ppm, with the exception of Weeks 5-8 in females; 750 ppm food utilization was significantly lower in males at Weeks 5-8 and 1-13 and in females at Weeks 1-4 and 1-13.
The differences noted in males at 100 ppm were isolated and there were no corresponding changes in body weight or food consumption. This was therefore considered not to be related to treatment.

Water consumption - Visual inspection indicated no observable differences between groups throughout the treatment period.

Ophthalmoscopy - There were no ophthalmoscopy findings seen which were considered to be related to consumption of diets containing SYN545192.

Clinical observations - There were no differences in the functional observation battery parameters that were considered to due to the consumption of diets containing SYN545192.

Motor activity -There were no differences in motor activity that were considered to be due to the consumption of diets containing SYN545192. Motor activity was noted to be significantly lower in males treated at 750 ppm in 1-5 minutes, and in all male treated groups at 6-10 minutes. Due to the isolated and inconsistent nature of these differences, and the absence of any significant overall changes, these findings were considered not to be related to treatment.

Detailed functional observations - There were no differences in detailed functional observation that could be positively attributed to the consumption of diets containing SYN545192. There was a statistical significant decrease in fore grip strength in females at 1500 ppm. Females treated at 750 or 1500 ppm had a statistically lower core body temperature. A lower core body temperature was also observed in males treated at 1500 ppm although did not achieve statistical significance.

Clinical Pathology

Haematology and coagulation - On analysis of the blood samples for haematology and coagulation, it was noted that a number of samples were either clotted or were insufficient and as such the number of samples analysed were: For males – N=6 in controls, N=7 in the low dose group, N=9 in the intermediate dose group and N=6 in the high dose group. For females – N=8 in controls, N=7 in the low dose group, N=8 in the intermediate dose group and N=3 in the high dose group.

There were no differences in haematology parameters which were considered to be related to treatment with SYN545192.

A statistically significant increase was noted at 1500 ppm in haemoglobin and mean cell volume in males. In females, red blood cell count was numerically higher. At 750 ppm, a statistical increase in basophils was noted in females while a statistical decrease in eosinophils was observed in males.

Due to the isolated nature of these differences and the lack of a dose related response, these were considered not to be related to treatment.

Table 4. Summary of hematology

ppm	0	100	750	1500
Males				
Hb (g/dL)	15.2 (±0.2)	15.4 (±0.5)	15.5 (±0.5)	15.9* (±0.5)
MCV (fL)	51.7 (±0.8)	51.5 (±0.9)	51.9 (±0.8)	53.5* (±1.1)
Eosinophilos	0.10 (±0.03)	0.13 (±0.02)	0.07* (±0.03)	0.07 (±0.02)
ppm	**0**	**100**	**750**	**1500**
Females				
RBC ($x10^{12}$/L)	7.46 (±0.29)	7.63 (±0.47)	7.66 (± 0.33)	8.10* (±0.29)
Baso (10^9/L)	0.00 (±0.01	0.01 (±0.01)	0.01* (±0.00)	0.01 (±0.01)

Data extracted from pages 69-72 of the study report

* Significantly different from control, p<0.05

** Significantly different from control, p<0.01

Clinical chemistry - Statistically significant decreases were noted in alkaline phosphatase and glucose in all animals at 1500 ppm, and in females only at 750 ppm. There was a statistically significant increase in urea at 750 ppm and above.

There were no other treatment related differences in clinical chemistry parameters. A small number of clinical chemistry values were statistically significantly higher or lower than their respective controls (alanine aminotransferase and aspartate aminotransferase in males, and cholesterol, total protein, albumin and albumin-globulin ratio in females), however due to the small magnitude of the difference and the absence of a dose response, they were considered not to be related to treatment.

Table 5. Summary of Clinical chemistry

ppm	0	100	750	1500
Males				
ALP (iu/L)	76 (±15)	73 (±13)	68 (±17)	57* (±18)
ALT (iu/L)	44 (± 8)	50 (± 8)	51 (± 6)	56** (± 10)
Urea (mmol/L)	5.8 (±0.7)	6.5 (±0.7)	6.9* (±0.8)	7.0** (±1.0)
Glu (mmol/L)	10.70 (1.13)	9.99 (0.85)	10.20 (0.76)	8.92** (0.65)
Ppm	**0**	**100**	**750**	**1500**
Females				
ALP (iu/L)	45 (±14)	36 (±9)	29** (±11)	33* (±8)
GLDH (iu/L)	3.8 (±0.7)	7.5** (±3.8)	3.6 (±1.9)	3.6 (±1.0)
Glu (mmol/L)	9.49 (±0.96)	9.88 (±0.92)	8.05** (±0.78)	7.71** (±0.65)
Chol (mmol/L)	1.4 (±0.2)	1.7 (±0.3)	2.0** (±0.3)	1.9** (±0.2)
TP (g/L)	72 (±5)	74 (±4)	70 (±2)	67** (±3)
Alb (g/L)	51 (±4)	51 (±5)	48 (±2)	44** (±2)
AG-R	2.3 (±0.3)	2.3 (±0.4)	2.2 (±0.3)	1.9* (±0.2)

Data extracted from pages 73-76 of the study report

* Significantly different from control, p<0.05

** Significantly different from control, p<0.01

Urinalysis - There were no differences seen in the urinalysis parameters that were considered to be related to treatment with SYN545192.

Organ weights - Following adjustment for bodyweight, covariant liver weights were noted to be higher in all treated animals compared to their respective controls achieving significance in males at 1500ppm and at 750 ppm in females. There was no statistically significant increase in females at 1500 ppm. A lower adjusted adrenal gland ($p<0.05$) and epididymide ($p<0.05$) weight in males and an increase in kidney ($p<0.05$) weight in females were noted in animals treated at 100 ppm. However, as there were no significant differences in high dose animals and no related microscopic changes in these organs the findings were considered to be incidental.

Table 6. Organ Weights (Covariance Analysis): Group Mean Values following 13 weeks of treatment

ppm	**0**	**100**	**750**	**1500**
Males				
Adrenals	0.0619 (±0.0031)	0.0512* (±0.0030)	0.0556 (±0.0030)	0.0592 (±0.0033)
Epididymides	1.3498 (±0.0417)	1.2134* (±0.0399)	1.3347 (±0.0398)	1.4096 (±0.0419)
Liver	13.56 (±0.44)	13.84 (±0.42)	15.04 (±0.42)	17.1** (±0.44)
ppm	**0**	**100**	**750**	**1500**
Females				
Kidneys	1.50 (±0.04)	1.63* (±0.04)	1.51 (±0.03)	1.44 (±0.05)
Liver	7.98 (±0.22)	8.48 (±0.22)	8.84* (0.19)	8.55 (±0.26)

Data extracted from pages 83-86 of the study report

* Significantly different from control, $p<0.05$

** Significantly different from control, $p<0.01$

Necropsy findings - Prominent liver lobulation was seen in one male dosed with 1500 ppm SYN545192. All other necropsy findings were typical of spontaneously arising background findings in rats of this age on this kind of study at Charles River, Edinburgh

Histological findings - Centrilobular hepatocyte hypertrophy of the liver was present in all male animals treated at 1500 ppm and 4/10 animals treated at 750 ppm. In females this finding was recorded in 4/10 animals from the high (1500 ppm) dose group only. All other histology findings were representative of the background pathology in rats of this age on this kind of study at Charles River, Edinburgh.

Discussion - Daily administration of SYN545192 for at least 90 consecutive days at 750 ppm and above resulted in a reduction in body weight, food consumption and food utilization.
Differences in functional observation battery parameters were noted in females treated at 1500 ppm (fore grip strength) and in females treated at 750 or 1500 ppm only (core temperature). The effects on fore grip strength are considered to be secondary to the significant reductions in mean body weights observed in females at 1500 ppm.

Statistically significant decreases were noted in alkaline phosphatase and glucose in both sexes at 1500 ppm, and in females only at 750 ppm. Additionally, there was a statistically significant increase in urea in males at 750 and 1500 ppm. In the absence of any associated microscopic changes or alterations in other clinical chemistry parameters indicative of target organ toxicity these findings are considered to be non-adverse.

Covariant liver weight, adjusted for bodyweight, was noted to be statistically significantly higher in males treated at 1500 ppm and in females treated at 750 ppm only.

Treatment with SYN545192 was associated with centrilobular hypertrophy of the liver in males treated at 750 ppm and above and in females treated at 1500 ppm.

Investigators' conclusions – "Dietary administration of SYN545192 to rats for at least 90 days was associated with in-life effects (reduced bodyweights, food consumption and food utilisation). Covariant liver weights were statistically higher in males treated at 1500 ppm and in females at 750 ppm. Centrilobular hepatocyte hypertrophy of the liver was noted at doses of 750 ppm and above in males and at 1500 ppm in females.

A clear No Observed Effect Level (NOEL) in this study was considered to be 100 ppm for both males and females equating to 7.6 mg/kg/day in males and 8.2 mg/kg/day in females."

Reviewer comments – Alanine aminotransferase and aspartate aminotransferase in males, and cholesterol, total protein, albumin and albumin-globulin ratio in females could to be treatment-related. Since some of these effects are observed from the concentration of 750 ppm and until at a concentration of 1500 ppm.

GLDH was increase for SYN545192 at 100 ppm in females. This changes was not believes to be an effect of treatment due to the lack of a dose response.

The hematology table shows a statistically significant decrease in eosinophils in the concentrations of 500 and 1500 ppm, however, this decrease at the concentration of 1500 ppm (0.07±0.02) is not marked as significant in the document.

Study deficiencies – None

Study Type: SYN545192 - 13 Week Dietary Toxicity Study in Mice; OPPTS 870.3100 (rodent); OECD 408, EU DIRECTIVE 96/54/EC B.26.

Test Material (purity): 97%

Citation: SYN545192 – 90 Day Dietary Study in mice. Charles River, Tranent Edinburgh, EH33 2NE UK. Laboratory report number: 30511. Study completion date: August 11, 2011.

Sponsor: Syngenta Crop Protection, LLC, USA.

Executive Summary: In a subchronic toxicity study, SYN545192 (97 % a.i.) was administered to CD-1 mice/sex/dose in diet at dose levels of 0, 100, 300 or 500 ppm for 90 days. Additional satellite groups of 4 male and 4 female animals were incorporated onto the study for toxicokinetic sampling and analysis.

In males, body weight loss was observed during the initial 4 days feeding at 300 ppm and after 7 days at the 500 ppm level. In females, body weight loss was observed during the initial 4 days feeding at 300 ppm and after 2 days at the 500 ppm level.

Statistically lower plasma triglyceride levels were noted in males at 300 and 500 ppm and statistically higher creatinine levels were observed in males treated at 500 ppm. A statistical significant increase in plasma calcium levels was noted in females dosed at 300 and 500 ppm. These findings were considered to be treatment related.

Treatment with SYN545192 was associated with distended large intestine in one animal of each sex at 300 ppm and 500 ppm. Minimal to moderate mucosal hyperplasia of the colon and/or rectum was observed in males and females treated at 500 ppm. Additionally, minimal to mild mucosal hyperplasia was seen in the colon and/or rectum of some males and females treated at 300 ppm.

The No Observed Effect Level (NOEL) for this study was considered to be 100 ppm for both sexes, equating to an average compound intake of 17.0 mg/kg/day in males and 20.9 mg/kg/day in females.

This subchronic toxicity study in the mouse is acceptable and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3100; OECD 408) in the mouse.

Materials and Methods

Materials

Test material:	SYN545192
Description:	Light beige powder
Lot/Batch #:	SMU9BP005
Purity:	97%
Vehicle:	Not reported
Positive control:	Not reported

Test species:	Mice	
Strain:	CD-1 (Crl:CD-1 (ICR))	
Sex:	59 female and 59 male	
Age at start:	7 weeks old	
Weight at start:	Males 27.9 to 41.0 g Females 24.3 to 32.6 g.	
Source:	Charles River UK Limited	
Housing:	Male animals were housed 1 per cage and female animals 2 per cage in suspended polycarbonate cages (overall dimensions 48 x 15 x 13 cm) with stainless steel grid tops and solid bottoms containing a separate stainless steel food hopper.	
Diet:	Rat and Mouse (modified) No. 1 Diet SQC Expanded (Ground) was supplied by Special Diets Services Limited, 1 Stepfield, Witham, Essex and available ad libitum.	
Water:	Animals had access to domestic mains water ad libitum throughout the study. The water used by Charles River is analysed at regular intervals for dissolved materials, heavy metals, pesticide residues, pH, nitrates and nitrites.	
Environmental conditions:	**Temperature:**	19-23°C
	Humidity:	40-70%
	Air changes:	15 air changes per hour
	Photoperiod:	Light hours were 0700-1900 hour
Acclimation:	3 weeks prior to the administration of experimental diets.	

Study design and methods

Study experimentation dates: Start: April 7, 2009 End: August 11, 2011

Animal assignment - Animals were allocated to 4 dose groups as and treated as follows:

Table 1. Study design

Group	SYN545192 Treatment (ppm)		Animals			
			Toxicity Study		Satellite Study	
			Male	Female	Male	Female
1	Control	0	1 - 10	41 – 50	81 - 84	97 - 100
2	Low	100	11 - 20	51 - 60	85 - 88	101 - 104
3	Intermediate	300	21 - 30	61 – 70	89 - 92	105 - 108
5	High	500	31 - 40	71 - 80	93 - 96	109 - 112

Diet preparation and analysis – A dietary stock premix at 5000 ppm was prepared first by making 200g of premix containing the total amount of test item required to produce the final concentration of diet requested of each dose group.

Diet formulations in the range of 100-500 ppm were prepared once every two weeks and stored at ambient temperature in the dark prior to administration to the animals.

Blank diet (without test item under investigation) was prepared for Control animals.

Prior to study commencement, stability data was generated by Charles River for 30 days for diet formulations stored at ambient temperature in the dark, in the concentration range of 10-5000 ppm under a separate study (Charles River Study No. 425169). In this study all diets were used in the period of established stability.

Results - Analyses of formulated diets sampled and prepared for use during the study indicated that following formulation, all concentrations were within -6.9% to -2.6% of the theoretical concentrations. The coefficient of variation for the triplicate samples analysed was 0.4% to 2.7%. All diet formulations were considered to be homogeneous and accurately formulated.
The result from the analyses of the control diet formulations indicated a found concentration of 0 ppm.

Statistics – Body weight, cumulative body weight gain, food consumption, food utilisation, haematology, and clinical chemistry data were analysed using a parametric ANOVA and pairwise comparisons made using the Dunnett's t-test distribution.

Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a twosided Dunnett's t-test, based on the error mean square in the analysis. All statistical tests were two sided. Summary statistics (mean, standard deviation and number of observations) are presented for organ weights as a percentage of body weight.

The following pairwise comparisons were performed:

Control vs Low Dose
Control vs Intermediate Dose
Control vs High Dose

Histopathology data were analysed using Fishers Exact Probability Test. Findings with multiple severities were analysed using a Mann-Whitney-U-test.
All statistical tests were two-sided and performed at the 5% and 1% significance level using in-house software. Males and females were analysed separately.

Methods

Observations – All animals were checked early morning and as late as possible each day for viability.

At least once each week, all animals received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.

Body weight - Body weights were recorded twice during pretrial, daily during the first 15 days and then twice weekly thereafter until the completion of treatment.

Food consumption and Food utilisation- The quantity of food consumed by each cage of animals was measured and recorded twice during pretrial, daily during the first 7 days and then twice weekly thereafter until the completion of treatment.

Clinical Pathology - Blood samples for haematology and clinical chemistry were collected from all surviving Toxicity Study animals via the orbital sinus under isoflurane anaesthesia, prior to terminal kill.

Hematology – 0.35 mL of whole blood was sampled into tubes containing EDTA and assayed for:

X	Haemoglobin	X	Neutrophils
X	Red Blood Cell Count	X	Lymphocytes
X	White Blood Cell Count	X	Monocytes
X	Mean Cell Volume	X	Eosinophils

X	Mean Cell Haemoglobin	X	Basophils
X	Mean Cell Haemoglobin Concentration	X	Large Unclassified Cells
X	Platelet Count	X	Haematocrit
X	Reticulocytes		

Clinical chemistry - 0.5 mL of whole blood was sampled into tubes containing lithium heparin which were centrifuged and assayed for:

X	Alkaline Phosphatase	X	Albumin Globulin Ratio
X	Alanine Aminotransferase	X	Phosphate
X	Aspartate Aminotransferase	X	Calcium
X	Creatinine	X	Creatine Phosphokinase
X	Urea	X	Gamma Glutamyl Transferase
X	Glucose	X	Total Bilirubin
X	Cholesterol	X	Sodium
X	Triglycerides	X	Potassium
X	Albumin	X	Chloride
X	Total Protein		
X	Globulin		

Terminal Studies

After at least 90 days of treatment all animals were killed in a randomised order by exposure to carbon dioxide and had their terminal body weight recorded followed by exsanguination.

Each animal was subject to a detailed necropsy under the guidance of a veterinary pathologist, who visited on the first day of necropsy and remained on call throughout the necropsy period. The necropsy consisted of a complete external and internal examination, which included body orifices (ears, nostrils, mouth, anus and vulva) and cranial, thoracic and abdominal cavities. All gross findings were recorded in terms of location(s), size (mm), shape, colour and number.

The organs marked ‘x’ in the ‘Weighed’ column in the table below were removed and weighed before preservation. Paired organs were weighed separately and the sum of the individual organs used for reporting purposes.

Representative samples of tissues listed in the table below were taken from all animals and fixed in 10% neutral buffered formalin, unless otherwise stated.

Tissues marked ‘x’ in the ‘Examined’ column, in the table below, were processed to paraffin wax block from all Toxicity study animals. Sections were cut 4-6 µm thick and stained with haematoxylin and eosin (H&E) from all animals in the Control and High dose group as well as colon, rectum and liver sections from Low and Intermediate dose groups, which were then subject to histopathological evaluation by the study pathologist.

Tissues collected	Weighed	Examined	Comments
Abnormal Tissue	-	x	-
Adrenal x 2	x	x	-
Aortic Arch	-	x	-
Blood smear	-	-	From animals killed prematurely
Brain	x	x	Forebrain, midbrain, cerebellum and pons.

Epididymis x 2	x	x	-
Eye x 2 .	-	x	Both eyes were fixed in Davidson's fluid. One only was processed and examined
Gastro-intestinal Tract: Stomach Duodenum Jejunum Ileum Caecum Colon Rectum	 - - - - - - -	 x x x x x x x	Opened at necropsy and mucosa examined. Peyers patches were sampled from small intestine.
Harderian Gland x 2 .	-	x	Both fixed in Davidson’s fluid. Only one processed and examined
Heart	x	x	-
Implant	-	-	For identification purposes.
Kidney x 2	x	x	A transverse section was taken from both kidneys and processed to paraffin wax block after 48h. A small section of duodenum was incorporated into each block.
Larynx	-	x	-
Liver + Gall Bladder	x	x	Liver sections were processed after 48 h. An additional section of duodenum was included with each liver section.
Lung	-	x	All lobes were examined, including mainstream bronchi.
Marrow Smear (femur) .	-	x	Smear air-dried and fixed in methanol.
Mesenteric Lymph Node	-	x	-
Nasal Cavity	-	x	-
Oesophagus	-	x	-
Optic Nerve x 2	-	x	Fix in Davidson's fluid. Only one examined.
Ovary x 2	x	x	-
Pancreas	-	x	-
Pharynx	-	x	-
Pituitary	-	x	-
Prostate	-	x	-
Sciatic Nerve	-	x	-
Seminal Vesicles	-	x	-
Skin + Mammary Gland	-	x	-
Spinal Cord	-	x	Cervical, midthoracic and lumbar regions.
Spleen	x	x	-

Sternum	-	x	Including bone marrow
Submandibular Lymph Node	-	x	-
Submaxillary (Mandibular) Salivary Gland (including sublingual)	-	x	Only one processed and examined.
Testis	x	x	Weighed individually, then fixed in Modified Davidson's fixative, trimmed after ca 18-24 h. Trimmed tissue then transferred to neutral buffered formalin prior to processing for examination.
Thigh Muscle	-	x	-
Thymus	x	x	-
Thyroid with Parathyroid x 2	-	x	-
Trachea	-	x	-
Urinary Bladder .	-	x	Contracted bladders distended with fixative; epithelial surface in animals which underwent histological evaluation were examined after fixation
Uterus (with Cervix)	x	x	-
Vagina	-	x	-

Results

Mortality - There were 3 high dose male animals prematurely removed from the study and they were killed on Days 73 (1 animal, toxicity study), 9 and 15 (2 animals, satellite study).

Animal of toxicity study was removed due to the overall clinical condition of the animal, with excess scratching, scabbed and damaged ears, scab on dorsal neck and agitated among the observations noted. Due to the nature of the histological findings (ulcerative dermatitis) the removal of the animal from the study was considered not to be related to treatment.

Two animals of satellite study showing weight loss.

Incidence of clinical observations - Clinical observations noted for both male and female treated animals included piloerection, rolling gait, staggering, circling, irregular respiration and softer than normal faeces. The incidence of soft faeces in males at 300 and 500 ppm suggests a relationship to treatment. However, for the other observations there was no discernable link between observations and dose level, and as such were considered to be incidental and not to be related to treatment.

Body weights and cumulative body weight change – A statistically significant body weight loss was noted in both sexes treated at 300 and 500 ppm following one days feeding with the treated diets.

In males, body weight loss was observed during the initial 4 days feeding at 300 ppm and after 7 days at the 500 ppm level. Although the animals subsequently gained weight the group mean body weights did not return to their starting values until after 21 days feeding at the 300 ppm level and 35 days at the 500 ppm level.

Table 1. Body weights (g) during 90 days of treatment

Group:		1	2	3	4
Test Item:		Control	SYN545192	SYN545192	SYN545192
Dosage (ppm):		0	100	300	500
Group / Sex		Day			
		0	7	14	21	28	31	38	49	56	63	70	77	84	91
1 M	Mean	37.3	36.5	37.6	39.1	40.1	40.3	40.9	44.4	45.0	47.0	47.0	48.4	49.6	50.6
	SD	2.0	2.6	3.0	3.7	3.5	3.7	3.9	5.0	4.9	5.6	6.2	6.8	6.9	7.7
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
2 M	Mean	34.3	34.4	36.1	37.5	38.5	40.1	40.1	42.3	42.2	44.2	44.6	45.8	45.6	47.7
	SD	3.2	3.3	3.6	3.9	4.1	4.4	4.7	5.5	5.2	5.6	6.1	6.4	6.6	6.7
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
3 M	Mean	35.2	32.7*	34.7	35.9	37.7	37.3	38.1	39.1	40.1	40.9*	41.7	42.0	42.8*	43.8
	SD	2.8	2.4	2.9	3.3	3.3	3.8	4.6	6.0	5.6	6.1	5.7	6.7	6.4	6.7
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
4 M	Mean	33.9**	30.0**	31.8**	32.4**	33.8**	32.8**	34.1**	34.8**	34.2**	34.8**	35.6**	37.0**	38.9**	38.4**
	SD	3.2	2.9	3.1	3.1	3.3	3.7	4.7	4.1	4.4	4.8	4.6	5.0	4.2	3.8
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10

		Days													
		0	7	14	21	28	31	38	49	56	63	70	77	84	91
1 F	Mean	28.1	28.6	30.3	31.7	32.5	33.9	34.6	37.3	35.8	37.5	38.4	40.7	41.6	42.0
	SD	1.4	1.7	2.0	1.9	1.4	2.1	2.5	3.7	4.2	3.4	5.2	4.9	4.5	6.3
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
2F	Mean	27.4	27.5	29.4	31.2	31.7	32.3	33.3	34.7	36.1	37.0	38.3	39.9	39.4	39.8
	SD	1.8	1.7	2.1	3.1	3.1	3.8	4.6	4.2	5.7	5.5	5.8	5.7	6.9	6.0
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
3F	Mean	28.8	27.8	29.9	31.5	31.9	31.6	33.3	34.1	34.0	34.2	35.9	37.0	37.4	37.6
	SD	2.0	1.6	2.2	2.8	2.8	3.4	4.6	4.7	3.8	4.5	5.3	6.3	6.0	5.3
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
4F	Mean	27.7	26.0**	27.9*	27.9*	30.0	29.8*	30.5	31.8*	30.8*	32.4	31.9*	34.5*	34.7*	35.1*

	SD	2.1	2.3	2.5	3.1	3.1	3.8	4.1	3.9	4.3	4.9	5.3	5.6	5.4	6.6
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Significantly different from Group 1: *=p<0.05, **=p<0.01

In females, body weight loss was observed during the initial 4 days feeding at 300 ppm and after 2 days at the 500 ppm level. The group mean body weights did not return to their starting values until after 11 days feeding at the 300 ppm level and 14 days at the 500 ppm level.

Body weight gain was occasionally statistically significantly lower at 300 ppm and considerably statistically significantly lower at 500 ppm over the period of the study compared to the controls.

Food consumption and food utilisation- Slight variances in food consumption were noted for both male and female treated animals, some of which were seen to be statistically significant when compared to their respective controls. On review of the food consumption as the study progressed, there appears to be a slight initial difference in the food consumed by the animals treated with 500 ppm diet. This is consistent with the initial body weight losses seen in this group.
Food utilisation over the study was noted to decrease as the treatment level increased for both sexes. This correlates with the body weight changes differences noted throughout the dose groups.

Blood analyses
Hematology – Slight variances in hematology were noted for both male and female treated animals.
Hemoglobin, Red Blood Cell, Haematocrit was slightly statistically decreases in the 100 ppm in males.
Monocytes were slightly statistically decreases in the 100 ppm in females.
Neutrophils were statistically increased in the 300 ppm in the male and female at 500 ppm.

Table 2. Summary of hematology

ppm	0	100	300	500
Females				
Monocytes (x10^9/L)	0.14 ± 0.07	0.06 ± 0.04**	0.09 ± 0.05	0.12 ± 0.04
Neutrophils	0.68 ± 0.29	0.53 ± 0.22	0.85 ± 0.43	1.22 ± 0.53**
ppm	**0**	**100**	**300**	**500**
Males				
Hemoglobin (g/dL)	13.8 ± 1.1	12.3 ± 1.3*	13.6 ± 1.4	14.2 ± 1.0
Red Blood (Cell x10^{12}/L)	9.28 ± 0.54	8.38 ± 0.65*	9.20 ± 0.90	9.63 ± 0.83
Haematocrit (L/L)	0.452 ± 0.030	0.406 ± 0.028*	0.445 ± 0.044	0.476 ± 0.039
Neutrophils	0.63 ± 0.42	0.56 ± 0.33	1.38 ± 0.99*	1.23 ± 0.63

Significantly different from Group 0: *=p<0.05, **=p<0.01.
Data extracted from pages 69-70 of the study report.

Clinical chemistry – A statistically significant higher plasma globulin and corresponding decrease in albumin globulin ratio were noted in both sexes at 500 ppm and in males only at 300 ppm. However, as the magnitude of these changes

was small and there were no corresponding changes in total protein or albumin concentrations, they are unlikely to be treatment related.

Statistically lower plasma triglyceride levels were noted in males at 300 and 500 ppm and statistically higher creatinine levels were observed in males treated at 500 ppm. A statistical significant increase in plasma calcium levels was noted in females dosed at 300 and 500 ppm.

Table 3. Summary of Clinical Chemistry

ppm	0	100	300	500
Males				
Triglyceride (mmol/L)	2.06 (±0.53)	1.47 (±0.49)	1.42 (±0.71)*	1.30 (±0.36)*
plasma globulin (g/L)	22 (±2)	23 (±1)	26 (±2)**	27 (±1)**
Albumin globulin ratio	1.6 (±0.2)	1.5 (±0.1)	1.4 (±0.2)**	1.2 (±0.1)**
Creatinine (µmol/L)	18 (±0)	18 (±0)	18 (±0)	19 (±3)*
ppm	**0**	**300**	**1000**	**3000**
Females				
plasma globulin (g/L)	20 (±1)	21 (±1)	22 (±2)	22 (±2)*
Albumin globulin ratio	1.9 (±0.2)	1.8 (±0.1)	1.7 (±0.2)	1.7 (±0.1)*
Cl^- (mmol/L)	111 (±4) n=6	116 (±2)* n=6	115 (±2) n=5	114 (±3) n=5
Ca^{2-} (mmol/L)	2.37 (0.07)	2.41 (0.06)	2.46 (0.08)*	2.49 (0.09)**

Significantly different from Group 0: *=p<0.05, **=p<0.01.
Data extracted from pages 71-74 of the study report.

Terminal Studies

Organ weight – Although there were statistically significant differences in absolute organ weights between treated and control animals, the majority of these statistical differences were no longer evident when subject to covariance analysis for body weight. A reduction in heart weight in males treated at 500 ppm was the only statistically significant finding after correction for terminal body weight, however this was at a reduced significance and there were no correlating microscopic findings in the heart. It is therefore concluded that the observed differences in organ weight were secondary to effects on body weight.

Necropsy findings - The large intestine was distended for one male and one female animal treated at 500 ppm, and one male and one female treated at 300 ppm.
There were no other noteworthy necropsy findings.– Enlargement of the adrenals was seen in 9 of the 10 high dose males. No other treatment-related findings were observed.

Histological findings – Treatment related histological findings were confined to the gastro-intestinal tract. Minimal to

moderate mucosal hyperplasia was found in the colon and/or rectum in most animals treated at 500 ppm. Minimal or mild mucosal hyperplasia was also found in the colon and/or rectum in some animals given SYN545192 at 300 ppm. In one male and one female the macroscopic finding of distended intestine was also observed at necropsy.

	Males				Females			
Dose Group/Treatment (ppm)	1 Control (0)	2 Low (100)	3 Intermediate (300)	4 High (500)	1 Control (0)	2 Low (100)	3 Intermediate (300)	4 High (500)
Colon Mucosal Hyperplasia	0/10	0/10	6/10*	8/10***	0/10	0/10	5/10*	9/10***
Rectum Mucosal Hyperplasia	0/10	0/10	3/10	4/10	0/10	0/10	5/10*	7/10**

Significantly different from the control: * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Discussion

Investigators' conclusions – "The No Observed Effect Level (NOEL) for this study was considered to be 100 ppm for both sexes, equating to an average compound intake of 17.0 mg/kg/day in males and 20.9 mg/kg/day in females. The NOEL is based on the occurrence of mortality in two animals at 500 ppm, initial body weight loss, decrease in body weight gain, an increased incidence of soft faeces in males, changes in clinical chemistry parameters and mucosal hyperplasia in the rectum and colon at 300 and 500 ppm".

Reviewer comments – Importantly, the concentrations of 300 and 500 ppm in the presence of hyperplasia 90 days exposure is observed in mice in both females and males. Es importante notar que en las concentraciones de 300 y 500 ppm se observa la presencia de hiperplasia en 90 días de exposición en ratones tanto en hembras y machos.

Study deficiencies –None.

Study Type: SYN545192 – 13-Week Oral (Capsule) Toxicity Study in the Beagle Dog; EPA OPPTS 870.3150 (1998); OECD 409 (1998); 88/302/EEC B.30 (1988)

Test Material (purity): 97%

Citation: SYN545192 – 13-Week Oral (Capsule) Toxicity Study in the Beagle Dog. Laboratory report number: C41606. Study completion date: July 7, 2010.

Sponsor: Syngenta Crop Protection, LLC, USA.

Executive Summary: In a 13-week oral toxicity study, SYN545192 was administered to 32 Beagle dogs, assigned to 4 groups each containing 4 males and 4 females. They were treated daily orally by capsule at dose levels of 0 (control group 1) or 30, 375 or 750 mg/kg (groups 2, 3 and 4, respectively). At the end of the study period, all animals were sacrificed, necropsied and examined *post mortem*. Histological examination was performed on an extended set of organs.

At the end of the treatment period all animals were weighed, sacrificed, necropsied and examined *post mortem*. Histological examination was performed on all scheduled organs and tissues from all animals on study including all gross lesions. All animals on study survived the scheduled study period. Daily administration of SYN545192 for 13 consecutive weeks resulted in no significant gross lesions that could be attributed to treatment with the test item. At 375 and 750 mg/kg/day, there was an initial body weight loss, decreased food consumption, decreased body weight gain and some effects on clinical biochemistry. There were no effects at 30 mg/kg/day which is considered to be the no observable adverse effect level (NOAEL) for this study.

This 13-Week Oral (Capsule) Toxicity Study in the Beagle Dog is acceptable and satisfies the guideline requirement for a toxicity study. 13-Week Oral (Capsule) Toxicity Study in the Beagle Dog; EPA OPPTS 870.3150 (1998); OECD 409 (1998); 88/302/EEC B.30 (1988)

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Materials and Methods

Materials

Test material:	SYN545192
Description:	Beige powder
Lot/Batch #:	SMU9BP005
Purity:	97%
Vehicle:	Not reported
Positive control:	Not reported
Test species:	Dog
Strain:	Beagle
Sex:	17 males and 17 females, of which 16 males and 16 females were selected for the study.
Age at start:	6.5 to 7 months
Weight at start:	5.6 to 9.7 kg
Source:	Harlan Laboratories

Housing:	The animals were housed Individually or group housing in pens with minimum of 2.0 square meters floor space per dog. Autoclaved standard softwood bedding, changed at least once weekly. Dogs were separated during the feeding period. Single housing was extended from day 12 to 14 in order to facilitate recording of the clinical signs and on days 1, 43 and 90 due to plasma sampling.	
Diet:	350 ± 1 g pelleted standard Kliba 3353 dog maintenance diet (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland), batch nos. 71/08, 80/08, 84/08 and 20/09. The diet was presented immediately after completion of dosing until day 8 of treatment phase and 3 hours after completion of dosing of all animals from day 9 onwards. Any remaining diet was withdrawn approximately three hours later. The day before clinical laboratory investigations food was withdrawn at the latest by approximately 14.00.	
Water:	Community tap water was supplied *ad libitum* by an automatic watering system.	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	22 ± 3 °C 30-70% 10-15 air changes per hour 12/12 hour light/dark cycle
Acclimation:	Under test conditions from delivery. A health check was performed on all animals to assure a satisfactory health status. The duration of the acclimation period was not reported.	

Study design and methods

Study experimentation dates: Start: 02 April 2009 End: 7 July 2010

Animal assignment

Table 1. Dose groups
Animals were allocated to dose groups as in the table below:

Group	SYN545192 Treatment (mg/kg/day)	Allocation	
		Male	Female
1 (Control)	0	1-4	17-20
2	30	5-8	21-24
3	375	9-12	25-28
4	750	13-16	29-32

Diet analysis and preparation – Results of representative analyses for contaminants in the diet were reviewed. There were no contaminants at levels that may have affected the conduct of the study. The results of these representative analyses are included in the raw data.
The appropriate amount of SYN545192 (in terms of the material as supplied) was weighed directly into gelatine capsules (Size 11, Torpac Inc., Fairfield, N.J., USA). The individual weights of test item required for daily administration was adjusted based on the most recently recorded body weight.

Statistics – Body weights, cumulative body weight gain, food consumption, hematology, clinical biochemistry, quantitative urinalysis values (e.g. specific gravity) and absolute organ weights were analyzed initially by a one-way

analysis of variance (ANOVA).
Organ weights were also analyzed by analysis of covariance (ANCOVA) on final body weight.
For all of the parameters evaluated initially by ANOVA or ANCOVA, Dunnett's test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA. The Dunnett's test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant, and statistical flags are presented in the tables of results in the report. Macropathology and micropathology incidence data were analyzed using Fisher's exact Test.

Methods

Clinical parameters - Blood and urine samples were collected from all animals at pre-test and during week 13. Additional samples were collected during week 4 (blood only), week 6 (urine only) and week 8 (blood only). The samples were collected from the first animal of each group in the order 4, 1, 3, 2 followed by the second animal in this group order until all animals had been sampled. The dogs were fasted overnight but allowed access to water *ad libitum*. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms. Blood samples were drawn from the jugular vein. Urine was collected into a specimen vial using a catheter.

Hematology and Biochemistry Parameters

The following parameters were examined.

hematology parameters	biochemistry parameters
Erythrocyte count	Glucose
Hemoglobin concentration	Urea
Hematocrit	Creatinine
Mean corpuscular volume	Bilirubin, total
Red cell volume distribution width	Cholesterol, total
Mean corpuscular hemoglobin concentration	Triglycerides
Mean corpuscular hemoglobin	Phospholipids
Hemoglobin concentration distribution width	Aspartate aminotransferase
Platelet count	Alanine aminotransferase
Reticulocyte count	Glutamate dehydrogenase
Reticulocyte maturity index	Creatine kinase
Total leukocyte count	Alkaline phosphatase
Differential leukocyte count	Gamma-glutamyl-transferase
Coagulation:	Calcium
Thromboplastin time	Phosphorus
Activated partial thromboplastin time	Sodium
	Potassium
	Chloride
	Protein, total

	Albumin
	Globulin
	Albumin/Globulin Ratio

Observations – All animals were checked at least twice daily for viability. Each animal was examined at least twice daily from pre-test for any change in behaviour, reaction to treatment or ill-health. A description of any abnormality was recorded from commencement of the pre-test period.

Ophthalmoscopic examination - Ophthalmoscopic examinations were performed on all animals at pre-test and in week 13. The examinations were performed using an ophthalmoscope after instillation of 0.5% tropicamide solution. Observations included the cornea, lens, conjunctiva, sclera, iris and fundus.

Food consumption – Food consumption was recorded daily from commencement of the pre-test period. The daily ration was weighed before and after feeding.

Body weight –The body weight of each animal was recorded once weekly from commencement of the pre-test period.

Urinalysis Parameters

The following urine parameters were determined:

Physical examination	Chemical examination
Color	pH
Appearance	Protein
Relative density	Glucose
	Ketone
	Urobilinogen
	Bilirubin
	Erythrocytes
	Leukocytes

Terminal Studies

Histotechnique

At the end of the treatment period all animals were anesthetized by intravenous injection of sodium pentobarbital and sacrificed by exsanguination. All organ and tissue samples were processed, embedded and cut at a nominal thickness of 4 micrometers and stained with hematoxylin and eosin.

Samples of the following tissues and organs were collected from all animals at necropsy:

Tissues/organs	Weighted	Collected	Examined
Adrenal glands	√	√	√
Aorta (abdominal)		√	√

Bone (femur and stifle joint)		√	
Bone and marrow (sternum)		√	√
Bone marrow smear (rib)		√	√
Brain - including sections of medulla/pons, cerebral and cerebellar cortex	√	√	√
Epididymides(fixed in Bouin's solution)	√	√	√
Esophagus		√	√
Eyes with optic nerve (fixed in Davidson’s solution)		√	√
Female mammary gland area		√	√
Gallbladder		√	√
Heart	√	√	√
Kidneys	√	√	√
Large intestine- cecum, colon, rectum		√	√
Larynx		√	
Liver	√	√	√
Lungs, instilled with formalin		√	√
Lymph nodes – retropharyngeal, mesenteric		√	√
Nasal passages		√	
Oviduct		√	√
Ovaries	√	√	√
Pancreas		√	√
Pharynx		√	
Pituitary gland		√	√
Prostate gland		√	√
Salivary glands – mandibular, parotid, sublingual		√	√
Sciatic nerve		√	√
Skeletal muscle		√	√
Skin and subcutaneous tissue (lateral thigh)		√	√
Small intestine – duodenum, jejunum, ileum		√	√
Spinal cord- cervical, midthoracic, lumbar segments		√	√
Spleen	√	√	√
Stomach		√	√
Testes (fixed in Bouin's solution)	√	√	√
Thymus		√	√
Thyroid (incl. parathyroid gland)	√	√	√
Trachea		√	√
Urinary bladder		√	√
Uterus with vagina and cervix	√	√	√
All gross lesions		√	√

Results

Mortality
All animals survived the scheduled treatment period

Body weights and cumulative body weight gain - Slight body weight loss between 0.4 and 0.9 kg was recorded in most of the animals during the first week of treatment at 375 and 750 mg/kg/day. There was a statistically significant decrease in mean body weight in males at 750 mg/kg/day from day 50 onwards. Reduced body weight gain was observed in males at 375 and 750 mg/kg/day from day 22 onwards and sporadically in females at 750 mg/kg/day between day 8 and the end of the study.
There were no effects on body weight in animals at 30 mg/kg/day. Slight fluctuations occasionally occurred but these were considered to reflect normal biological variation.

Food consumption - Mean food consumption was reduced in animals at 375 mg/kg/day and 750 mg/kg/day during the first two weeks of treatment. Food consumption improved in all animals after change of the feeding regimen (feeding 3 hours after completion of dosing instead of immediately after dosing).
There were no effects on food consumption in animals treated at 30 mg/kg/day.

Clinical observations - In animals of all treated groups it was frequently observed: Vomiting of mucus, gelatin capsule or feed. Incidence per animal and the number of animals affected increased with increasing dose levels. After changing the feeding regimen, the incidence of vomiting decreased. White particles were observed in several animals of all test-item treated groups. These were considered to represent remainders of the test item.
Tremor was observed twice in one male treated at 375 mg/kg/day. As this finding was not observed in any animal treated at 750 mg/kg/day, it is considered not to be treatment related.
A single occasion of salivation was seen in two females at 375 mg/kg/day. Salivation was also recorded in all females after treatment at 750 mg/kg/day.
Feces containing mucus or yellow stained feces were recorded in several animals treated at 375 mg/kg/day. Feces containing mucus or yellow particles or yellow stained feces were observed in most of the animals of the group treated at 750 mg/kg/day. As loose and watery feces were seen in animals of all groups, including controls, these observations are considered not to be related to treatment.

Ophthalmoscopic examination - There were no findings during ophthalmoscopic examination that were considered to be related to treatment with the test item.

Clinical biochemistry - increased plasma triglyceride values were recorded in the males and some females during the whole treatment period at 750 mg/kg/day. Decreased plasma calcium values were observed
in males at 375 and 750 mg/kg/day during week 8 and 13. There were no other changes which were considered to be related to treatment with the test item. Some intergroup variations occasionally achieved statistical significance, but these did not show a relationship to dose level, reflected differences which were present during pretest or in the controls, or were within the range of the historical control values.

Hematology
There were no differences in hematology parameters considered to be related to treatment with SYN545192. Some intergroup variations occasionally achieved statistical significance, but these did not show a relationship to dose level or reflected differences which were present during pretest and were considered to reflect normal biological variation.

Urinalysis - There were no differences seen in the urinalysis parameters that were considered to be related to treatment with SYN545192.

Organ weights - There were no changes in organ weights which were considered to be related to treatment with the test item. Some intergroup variations occasionally achieved statistical significance, but these did not show a relationship to dose level and/or were considered to reflect normal biological variation.

Necropsy findings - There were no necropsy findings recorded which were considered to be related to treatment with the test item. All findings were considered to be incidental and commonly occur in dogs of this strain and age under the experimental conditions used in this study.

Histological findings - There were no histological findings that were considered to be related to treatment with the test item. All the findings noted are commonly seen in animals of this age and strain under the conditions of this study.

Discussion - The purpose of this study was to assess the toxicity of SYN545192 when administered orally (by capsule) once a day to Beagle dogs for a period of 13 weeks.
Daily administration of SYN545192 at 750 mg/kg/day resulted in a reduction in body weight in the first week of the study; reduced food consumption, reduced body weight gain, and minor changes in clinical chemistry parameters salivation. Vomiting of feed, fluid, capsule or mucus as well as feces containing mucus were also observed. Similar effects were also seen at 375 mg/kg/day; transient reduced food consumption, loss of body weight in the first week of the study, reduced body weight in the males and minor changes in clinical biochemistry in males only. There were no effects after treatment at 30 mg/kg/day.

Investigators' conclusions – At 375 and 750 mg/kg/day, there was an initial body weight loss, decreased food consumption, decreased body weight gain and some effects on clinical biochemistry. There were no effects at 30 mg/kg/day which is considered to be the no effect level (NOEL) for this study.

Reviewer comments –This toxicity oral study is classified as acceptable. This study satisfies the guideline requirements (EPA OPPTS 870.4100 (1998); OECD 452 (1981); 88/303/EEC (1988)). This reviewer noted that allocation duration was not specified in this study; however, it does not affect its validity.
Additionally, we noted that a pathology phase report is provided separately, in which the NOEL was established at 750 mg/kg

Study deficiencies – We found no deficiencies

Study Type: SYN545192 – 13-Week Oral (Capsule) Toxicity Study in the Beagle Dog; EPA OPPTS 870.3150 (1998); OECD 409 (1998); 88/302/EEC B.30 (1988)

Test Material (purity): 97%

Citation: SYN545192 – 13-Week Oral (Capsule) Toxicity Study in the Beagle Dog. Laboratory report number: C41606. Study completion date: July 7, 2010.

Sponsor: Syngenta Crop Protection, LLC, USA.

Executive Summary: In a 13-week oral toxicity study, SYN545192 was administered to 32 Beagle dogs, assigned to 4 groups each containing 4 males and 4 females. They were treated daily orally by capsule at dose levels of 0 (control group 1) or 30, 375 or 750 mg/kg (groups 2, 3 and 4, respectively). At the end of the study period, all animals were sacrificed, necropsied and examined *post mortem*. Histological examination was performed on an extended set of organs.

At the end of the treatment period all animals were weighed, sacrificed, necropsied and examined *post mortem*. Histological examination was performed on all scheduled organs and tissues from all animals on study including all gross lesions. All animals on study survived the scheduled study period. Daily administration of SYN545192 for 13 consecutive weeks resulted in no significant gross lesions that could be attributed to treatment with the test item.At 375 and 750 mg/kg/day, there was an initial body weight loss, decreased food consumption, decreased body weight gain and some effects on clinical biochemistry. There were no effects at 30 mg/kg/day which is considered to be the no effect level (NOEL) for this study.

This 13-Week Oral (Capsule) Toxicity Study in the Beagle Dog is acceptable and satisfies the guideline requirement for a toxicity study. 13-Week Oral (Capsule) Toxicity Study in the Beagle Dog; EPA OPPTS 870.3150 (1998); OECD 409 (1998); 88/302/EEC B.30 (1988)

EPA Conclusion: LOAEL = 250 mg/kg/day based on reduced body weight, salivation, vomiting and feces containing mucus. NOAEL = 25 mg/kg/day.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Materials and Methods

Materials

Test material:	SYN545192
Description:	Beige powder
Lot/Batch #:	SMU9BP005

Purity:	97%	
Vehicle:	Not reported	
Positive control:	Not reported	
Test species:	Dog	
Strain:	Beagle	
Sex:	17 males and 17 females, of which 16 males and 16 females were selected for the study.	
Age at start:	6.5 to 7 months	
Weight at start:	5.6 to 9.7 kg	
Source:	Harlan Laboratories	
Housing:	The animals were housed Individually or group housing in pens with minimum of 2.0 square meters floor space per dog. Autoclaved standard softwood bedding, changed at least once weekly. Dogs were separated during the feeding period. Single housing was extended from day 12 to 14 in order to facilitate recording of the clinical signs and on days 1, 43 and 90 due to plasma sampling.	
Diet:	350 ± 1 g pelleted standard Kliba 3353 dog maintenance diet (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland), batch nos. 71/08, 80/08, 84/08 and 20/09. The diet was presented immediately after completion of dosing until day 8 of treatment phase and 3 hours after completion of dosing of all animals from day 9 onwards. Any remaining diet was withdrawn approximately three hours later. The day before clinical laboratory investigations food was withdrawn at the latest by approximately 14.00.	
Water:	Community tap water was supplied *ad libitum* by an automatic watering system.	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	22 ± 3 °C 30-70% 10-15 air changes per hour 12/12 hour light/dark cycle
Acclimation:	Under test conditions from delivery. A health check was performed on all animals to assure a satisfactory health status. The duration of the acclimation period was not reported.	

Study design and methods

Study experimentation dates: Start: 02 April 2009 End: 7 July 2010

Animal assignment

Table 1. Dose groups

Animals were allocated to dose groups as in the table below:

Group	SYN545192 Treatment (mg/kg/day)	Allocation	
		Male	Female
1 (Control)	0	1-4	17-20
2	30	5-8	21-24
3	375	9-12	25-28
4	750	13-16	29-32

Diet analysis and preparation – Results of representative analyses for contaminants in the diet were reviewed. There were no contaminants at levels that may have affected the conduct of the study. The results of these representative analyses are included in the raw data.
The appropriate amount of SYN545192 (in terms of the material as supplied) was weighed directly into gelatine capsules (Size 11, Torpac Inc., Fairfield, N.J., USA). The individual weights of test item required for daily administration was adjusted based on the most recently recorded body weight.

Statistics – Body weights, cumulative body weight gain, food consumption, hematology, clinical biochemistry, quantitative urinalysis values (e.g. specific gravity) and absolute organ weights were analyzed initially by a one-way analysis of variance (ANOVA).
Organ weights were also analyzed by analysis of covariance (ANCOVA) on final body weight.
For all of the parameters evaluated initially by ANOVA or ANCOVA, Dunnett's test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA. The Dunnett's test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant, and statistical flags are presented in the tables of results in the report. Macropathology and micropathology incidence data were analyzed using Fisher's exact Test.

Methods

Clinical parameters - Blood and urine samples were collected from all animals at pre-test and during week 13. Additional samples were collected during week 4 (blood only), week 6 (urine only) and week 8 (blood only). The samples were collected from the first animal of each group in the order 4, 1, 3, 2 followed by the second animal in this group order until all animals had been sampled. The dogs were fasted overnight but allowed access to water *ad libitum*. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms. Blood samples were drawn from the jugular vein. Urine was collected into a specimen vial using a catheter.

Hematology and Biochemistry Parameters

The following parameters were examined.

hematology parameters	biochemistry parameters
Erythrocyte count	Glucose
Hemoglobin concentration	Urea
Hematocrit	Creatinine
Mean corpuscular volume	Bilirubin, total
Red cell volume distribution width	Cholesterol, total
Mean corpuscular hemoglobin concentration	Triglycerides
Mean corpuscular hemoglobin	Phospholipids
Hemoglobin concentration distribution width	Aspartate aminotransferase
Platelet count	Alanine aminotransferase
Reticulocyte count	Glutamate dehydrogenase

Reticulocyte maturity index	Creatine kinase
Total leukocyte count	Alkaline phosphatase
Differential leukocyte count	Gamma-glutamyl-transferase
Coagulation:	Calcium
Thromboplastin time	Phosphorus
Activated partial thromboplastin time	Sodium
	Potassium
	Chloride
	Protein, total
	Albumin
	Globulin
	Albumin/Globulin Ratio

Observations – All animals were checked at least twice daily for viability. Each animal was examined at least twice daily from pre-test for any change in behaviour, reaction to treatment or ill-health. A description of any abnormality was recorded from commencement of the pre-test period.

Ophthalmoscopic examination - Ophthalmoscopic examinations were performed on all animals at pre-test and in week 13. The examinations were performed using an ophthalmoscope after instillation of 0.5% tropicamide solution. Observations included the cornea, lens, conjunctiva, sclera, iris and fundus.

Food consumption – Food consumption was recorded daily from commencement of the pre-test period. The daily ration was weighed before and after feeding.

Body weight –The body weight of each animal was recorded once weekly from commencement of the pre-test period.

Urinalysis Parameters

The following urine parameters were determined:

Physical examination	Chemical examination
Color	pH
Appearance	Protein
Relative density	Glucose
	Ketone
	Urobilinogen
	Bilirubin
	Erythrocytes
	Leukocytes

Terminal Studies

Histotechnique

At the end of the treatment period all animals were anesthetized by intravenous injection of sodium pentobarbital and sacrificed by exsanguination. All organ and tissue samples were processed, embedded and cut at a nominal thickness of 4 micrometers and stained with hematoxylin and eosin.

Samples of the following tissues and organs were collected from all animals at necropsy:

Tissues/organs	Weighted	Collected	Examined
Adrenal glands	√	√	√
Aorta (abdominal)		√	√
Bone (femur and stifle joint)		√	
Bone and marrow (sternum)		√	√
Bone marrow smear (rib)		√	√
Brain - including sections of medulla/pons, cerebral and cerebellar cortex	√	√	√
Epididymides(fixed in Bouin's solution)	√	√	√
Esophagus		√	√
Eyes with optic nerve (fixed in Davidson’s solution)		√	√
Female mammary gland area		√	√
Gallbladder		√	√
Heart	√	√	√
Kidneys	√	√	√
Large intestine- cecum, colon, rectum		√	√
Larynx		√	
Liver	√	√	√
Lungs, instilled with formalin		√	√
Lymph nodes – retropharyngeal, mesenteric		√	√
Nasal passages		√	
Oviduct		√	√
Ovaries	√	√	√
Pancreas		√	√
Pharynx		√	
Pituitary gland		√	√
Prostate gland		√	√
Salivary glands – mandibular, parotid, sublingual		√	√
Sciatic nerve		√	√
Skeletal muscle		√	√
Skin and subcutaneous tissue (lateral thigh)		√	√
Small intestine – duodenum, jejunum, ileum		√	√
Spinal cord- cervical, midthoracic, lumbar segments		√	√
Spleen	√	√	√

Stomach		√	√
Testes (fixed in Bouin's solution)	√	√	√
Thymus		√	√
Thyroid (incl. parathyroid gland)	√	√	√
Trachea		√	√
Urinary bladder		√	√
Uterus with vagina and cervix	√	√	√
All gross lesions		√	√

Results

Mortality
All animals survived the scheduled treatment period

Body weights and cumulative body weight gain - Slight body weight loss between 0.4 and 0.9 kg was recorded in most of the animals during the first week of treatment at 375 and 750 mg/kg/day. There was a statistically significant decrease in mean body weight in males at 750 mg/kg/day from day 50 onwards. Reduced body weight gain was observed in males at 375 and 750 mg/kg/day from day 22 onwards and sporadically in females at 750 mg/kg/day between day 8 and the end of the study.
There were no effects on body weight in animals at 30 mg/kg/day. Slight fluctuations occasionally occurred but these were considered to reflect normal biological variation.

Food consumption - Mean food consumption was reduced in animals at 375 mg/kg/day and 750 mg/kg/day during the first two weeks of treatment. Food consumption improved in all animals after change of the feeding regimen (feeding 3 hours after completion of dosing instead of immediately after dosing).
There were no effects on food consumption in animals treated at 30 mg/kg/day.

Clinical observations - In animals of all treated groups it was frequently observed: Vomiting of mucus, gelatin capsule or feed. Incidence per animal and the number of animals affected increased with increasing dose levels. After changing the feeding regimen, the incidence of vomiting decreased. White particles were observed in several animals of all test-item treated groups. These were considered to represent remainders of the test item.
Tremor was observed twice in one male treated at 375 mg/kg/day. As this finding was not observed in any animal treated at 750 mg/kg/day, it is considered not to be treatment related.
A single occasion of salivation was seen in two females at 375 mg/kg/day. Salivation was also recorded in all females after treatment at 750 mg/kg/day.
Feces containing mucus or yellow stained feces were recorded in several animals treated at 375 mg/kg/day. Feces containing mucus or yellow particles or yellow stained feces were observed in most of the animals of the group treated at 750 mg/kg/day. As loose and watery feces were seen in animals of all groups, including controls, these observations are considered not to be related to treatment.

Ophthalmoscopic examination - There were no findings during ophthalmoscopic examination that were considered to be related to treatment with the test item.

Clinical biochemistry - increased plasma triglyceride values were recorded in the males and some females during the whole treatment period at 750 mg/kg/day. Decreased plasma calcium values were observed

in males at 375 and 750 mg/kg/day during week 8 and 13. There were no other changes which were considered to be related to treatment with the test item. Some intergroup variations occasionally achieved statistical significance, but these did not show a relationship to dose level, reflected differences which were present during pretest or in the controls, or were within the range of the historical control values.

Hematology
There were no differences in hematology parameters considered to be related to treatment with SYN545192. Some intergroup variations occasionally achieved statistical significance, but these did not show a relationship to dose level or reflected differences which were present during pretest and were considered to reflect normal biological variation.

Urinalysis - There were no differences seen in the urinalysis parameters that were considered to be related to treatment with SYN545192.

Organ weights - There were no changes in organ weights which were considered to be related to treatment with the test item. Some intergroup variations occasionally achieved statistical significance, but these did not show a relationship to dose level and/or were considered to reflect normal biological variation.

Necropsy findings - There were no necropsy findings recorded which were considered to be related to treatment with the test item. All findings were considered to be incidental and commonly occur in dogs of this strain and age under the experimental conditions used in this study.

Histological findings - There were no histological findings that were considered to be related to treatment with the test item. All the findings noted are commonly seen in animals of this age and strain under the conditions of this study.

Discussion - The purpose of this study was to assess the toxicity of SYN545192 when administered orally (by capsule) once a day to Beagle dogs for a period of 13 weeks.
Daily administration of SYN545192 at 750 mg/kg/day resulted in a reduction in body weight in the first week of the study; reduced food consumption, reduced body weight gain, and minor changes in clinical chemistry parameters salivation. Vomiting of feed, fluid, capsule or mucus as well as feces containing mucus were also observed. Similar effects were also seen at 375 mg/kg/day; transient reduced food consumption, loss of body weight in the first week of the study, reduced body weight in the males and minor changes in clinical biochemistry in males only. There were no effects after treatment at 30 mg/kg/day.

Investigators' conclusions – At 375 and 750 mg/kg/day, there was an initial body weight loss, decreased food consumption, decreased body weight gain and some effects on clinical biochemistry. There were no effects at 30 mg/kg/day which is considered to be the no effect level (NOEL) for this study.

Reviewer comments –This toxicity oral study is classified as acceptable. This study satisfies the guideline requirements (EPA OPPTS 870.4100 (1998); OECD 452 (1981); 88/303/EEC (1988)). This reviewer noted that allocation duration was not specified in this study; however, it does not affect its validity.
Additionally, we noted that a pathology phase report is provided separately, in which the NOEL was established at 750 mg/kg

EPA Conclusion: LOAEL = 250 mg/kg/day based on reduced body weight, salivation, vomiting and feces containing mucus. NOAEL = 25 mg/kg/day.

Study deficiencies – We found no deficiencies

B.6.3 SYN545192 – 28-Day Dermal Toxicity (Semi-Occlusive) Study in the Wistar Rat

DERMAL TOXICITY

Study Type: SYN545192 – 28-Day Dermal Toxicity (Semi-Occlusive) study in the Wistar Rat. EPA OPPTS 870.3200; OECD 410 (1981).

Test Material: Content of SYN545192 97%

Synonyms: SYN545192

Citation: SYN545192 – 28-Day Dermal Toxicity (Semi-Occlusive) study in the Wistar Rat**.** Harlan Laboratories Ltd. Zelgliweg 1, 4452 Itingen / Switzerland. Laboratory report number: C72048. Study Completion Date: January 16, 2012.

Sponsor: Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

Report Number: C72048

Executive Summary – A 28-Day Dermal Toxicity (Semi-Occlusive) study was conducted with WIST (SPF) rats to determine the dermal toxicity of SYN545192.
SYN545192 was administered by bandage to the skin once daily at doses of 100, 300 and 1000 mg/kg/day. The control group received the bandage with vehicle (aqueous 0.5% CMC) only. Rats were treated for a period of 28 days.

The animals were observed for clinical signs once daily during both, acclimatization and the treatment period. Forelimb and hind limb grip strength, locomotor activity, food consumption, body weight and body weight were determined.
Animals were examined during the acclimatization period and during the final week of the study for ophthalmic abnormalities. Hematology, biochemistry, and urine parameters were also determined.
Animals were necropsied and organs were weighted. Tissue samples were examined, processed, embedded and cut at an approximate thickness of 2 - 4 micrometers and stained with hematoxylin and eosin.

All animals survived the scheduled treatment period. General daily clinical observations, weekly detailed behavioural observations, measurement of grip strength and locomotor activity, periodic measurements of food consumption and body weight, ophthalmoscopic examination, and measurement of clinical pathology parameters did not reveal any test item-related effects. No test item-related differences in organ weights, macroscopic findings, or microscopic findings were present.

Under the conditions of this study, dermal administration of SYN545192 to Wistar rats at doses of 100, 300 and 1000 mg/kg/day for 6 hours each day for 5 days weekly over a period of 28 days resulted in no toxicologically significant findings.
Based on the results of this study a no-observed effect level (NOEL) was determined to be 1000 mg/kg/day.

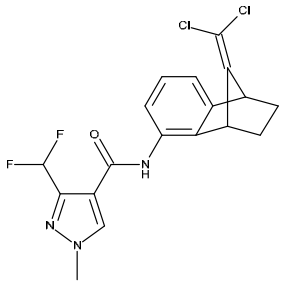
Compliance: Signed and dated GLP (Good Laboratory Practise), Quality Assurance, Flagging and Data

Confidentiality statements were provided.

US EPA: The study is classified as Acceptable/Guideline. The Agency concurs that the study NOAEL is 1000 mg/kg/day.

Materials and Methods

Materials

Test material:	SYN545192 	
Physical Description:	Beige powder	
Lot/Batch #:	SMU9BP005	
Purity:	97.0%	
Vehicle:	0.5% Carboxymethylcellulose (CMC; high viscosity grade)	
EPSL Reference Number:		
Test species:	Rat	
Strain:	RccHan: WIST(SPF)	
Sex: **Age at dosing:**	41 Females and 41 males 8 weeks	
Weight at dosing:	Males: 204.5 to 242.0 g (mean: 217.4 g) Females: 154.4 to 189.3 g (mean: 167.8 g)	
Source: **Housing:**	Harlan Laboratories, Inc. Individually in Makrolon type-3 cages with wire mesh tops and standard softwood bedding	
Diet:	Pelleted standard Kliba Nafag 3433 rodent maintenance diet	
Water:	Community tap-water from Itingen was available *ad libitum* in water bottles.	
Environmental conditions:	**Temperature:** **Humidity:** **Ventilation:** **Photoperiod:**	22 ± 3 °C; 30 - 70% 10-15 air exchanges/hour 12-hour light/dark cycle
Acclimation:	6 days	

Study design and methods

Study experimentation dates - Start: November 06, 2009 End: January 16, 2012

Treatment

Treatment was epidermal on the clipped intact skin of the back, on an exposed area of approximately 10% of the total body surface. The fur on the back of each rat was closely clipped as necessary exposing an area of approximately 25 cm² (approximately 10% of the total body surface).

Dosing

The animals received a daily dose of the test item as follows:

Group 1 (10 males and 10 females): 0 mg/kg/day
Group 2 (10 males and 10 females): 100 mg/kg/day
Group 3 (10 males and 10 females): 300 mg/kg/day
Group 4 (10 males and 10 females): 1000 mg/kg/day

Treatment period consisted in 28 days - comprising 4 cycles of 5 days per week (6 hours per day) test item application and 2 days per week without treatment.

Allocation and dose levels

Allocation and dose levels	**Group 1 (control)**	**Group 2**	**Group 3**	**Group 4**
mg/kg/day	**0**	**100**	**300**	**1000**
Males	1-10	11-20	21-30	31-40
Females	41-50	51-60	61-70	71-80

The following hematology and biochemistry parameters were determined

hematology parameters	biochemistry parameters
Erythrocyte count	Glucose
Hemoglobin	Urea
Hematocrit	Creatinine
Mean corpuscular volume	Bilirubin, total
Mean corpuscular hemoglobin	Cholesterol, total
Mean corpuscular hemoglobin concentration	Triglycerides
Reticulocyte count	Aspartate aminotransferase
Erythrocyte morphology	Alanine aminotransferase
Hemoglobin distribution width	Alkaline phosphatase
Differential leukocyte count:	Sodium

Neutrophils	Potassium
Eosinophils	Chloride
Basophils	Calcium
Lymphocytes	Phosphorus
Monocytes	Protein, total
Large unstained cells	Albumin
Platelet count	Globulin
Leukocyte count, total	Albumin/Globulin ratio
Clotting (PT and PTT)	

The following parameters were determined in Urinalysis

Urialysis parameters	Chemical examination
Urine volume (18 hour)	pH value
Specific gravity (relative density)	Nitrite
Color	Protein
Appearance	Glucose
	Ketones
	Urobilinogen
	Bilirubin
	Leukocytes

Samples of the following tissues and organs were collected from all animals at necropsy:

Tissues/organs	Weighted	Collected	Examined
Adrenal glands	√	√	√
Aorta		√	√
Bone (sternum, femur including joint)		√	√
Bone marrow (sternum, femur)		√	√
Brain - including section of medulla/pons, cerebral and cerebellar cortex	√	√	√
Cecum		√	√
Colon		√	√
Duodenum		√	√
Epididymides(fixed in Bouin's solution)	√	√	√
Esophagus		√	√
Eye with optic nerve (fixed in Davidson’s solution)		√	√
Harderian gland (fixed in Davidson’s solution)		√	√
Heart including auricles	√	√	√
Ileum, with Peyer’s patches		√	√
Jejunum with Peyer’s patches		√	√
Kidneys	√	√	√
Larynx		√	√
Lacrimal gland, exorbital		√	√

Liver	√	√	√
Lungs, filled w/formalin at necropsy		√	√
Lymph nodes – mesenteric and mandibular and popliteal		√	√
Mammary gland area		√	√
Nasal cavity		√	√
Ovaries	√	√	√
Pancreas		√	√
Pharynx		√	√
Pituitary gland		√	√
Prostate gland including coagulating glands		√	√
Rectum		√	√
Salivary glands- mandibular, sublingual		√	√
Sciatic nerve		√	√
Seminal vesicles		√	√
Skeletal muscle		√	√
Skin (treated and untreated)		√	√
Spinal cord- cervical, midthoracic, lumbar		√	√
Spleen	√	√	√
Stomach		√	√
Testes (fixed in Bouin's solution)	√	√	√
Thymus	√	√	√
Thyroid (incl. parathyroid gland, if possible)		√	√
Tongue		√	√
Trachea		√	√
Ureter		√	√
Urinary bladder, filled w/formalin at necropsy		√	√
Uterus		√	√
Vagina		√	√
All gross lesions		√	√

Statistical Analysis - glm was used for the diagnostic analysis of variance according to Bartlett, and prox mix was applied for the Dunnett's test. All analyses were two-tailed for significance levels of 5% and 1%, and all means were presented with standard deviations.
Body weights, cumulative body weight gain, food consumption, clinical pathology values (hematology, clinical chemistry, and urinalysis) and absolute organ weights were analyzed initially by a one-way analysis of variance (ANOVA) and by analysis of covariance (ANCOVA) on final body weight. For all of the parameters evaluated initially by ANOVA or ANCOVA, the Dunnett's test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA. Parameters that yield discontinuous or descriptive data were analyzed by Fisher's Exact Test (instead of the Mann-Withney U-test). Macropathology and micropathology incidence data were analyzed using Fisher's exact test.

Results and Discussion

Mortality

There was no mortality in the study. All animals survived their scheduled study period.

Clinical signs

No effects body weights, hematological, biochemical and urinalysis parameters, grip strength, locomotor activity or food consumption, were observed.

No test item-related clinical signs were observed.
A slight erythema (grade 1) was present at the application site on two days only in the second half of week 3 in one male dosed at 100 mg/kg. In one female of the control group the transient occurrence of a weakened condition was observed. In addition, one female of the low dose group was found with a dark nose from days 4 to 6. Given the low number of affected animals and the absence of this observation at the higher dose levels, these findings were considered to represent individual biological variations in the physiological condition rather than test item-related effects

Macroscopic findings

No treatment-related macroscopic findings were noted in this study. A few non item-related macroscopic findings included: dark red foci in the thymus or stomach, testes and epididymides reduced in size in one control rat, pelvic dilation of the kidneys, uterine dilation or dark red discoloration of the ovaries.

Microscopic findings

There were no microscopic findings present in rats treated with SYN545192 that could be considered to represent a test item-related effect. Some minor microscopic findings included pelvic dilation, tubular basophilia and corticomedullary mineralization (in females) of the kidneys, mixed cell infiltration in the liver, thymic congestion and mild lymphoid depletion, or uterine dilation.

Investigator's Conclusions – Under the conditions of this study, dermal administration of SYN545192 to Wistar rats at doses of 100, 300 and 1000 mg/kg/day for 6 hours each day for 5 days weekly over a period of 28 days resulted in no toxicologically significant findings.
Based on the results of this study a no-observed effect level (NOEL) was determined to be 1000 mg/kg/day.

Reviewer's Conclusions -This Dermal Toxicity (Semi-Occlusive) study in the Wistar Rat is classified acceptable.

Deficiencies – None

US EPA: The study is designated as Acceptable/Guideline. The Agency concurs that the study NOAEL is 1000 mg/kg/day. There was no effect on coagulation (PT or PTT) in the study.

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SYN545192 tech

B.6. Toxicology and metabolism

B.6.4 Genotoxicity

Study type: SYN545192 tech. – *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay; OECD 471 (1997), OPPTS 870.5100 (1998), EC 2000/32 (2000).

Test Material (purity): SYN545192 tech (97.6 %)

Synonyms: SYN545192 tech

Sponsor: Syngenta Crop Protection, Inc. Greensboro, USA.

Report Number: 1431400

Executive Summary: In a reverse gene mutation assay in bacteria, strains TA1535, TA98, TA1537 and TA100 of *Salmonella typhimurium* and strains *WP2 uvrA* pKM101 and WP2 pKM101 of *Escherichia coli* were exposed to SYN545192 tech (97.6 %) in DMSO at concentrations 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate with S9 mix.

Minor toxic effects were observed in strain TA 1537 at 2500 and 5000 µg /plate with metabolic activation in experiment I. No toxic effects were observed in the remaining strains and in experiment II.
Precipitation of the test item was observed in the overlay agar in the test tubes and on the incubated agar plates from 1000 - 5000 µg /plate in both experiments. The undissolved particles had no influence on the data recording.
No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with SYN546482 at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.
The laboratory´s historical control range was slightly exceeded in the solvent control of strain WP2 pKM101 with and without metabolic activation in experiment I, and without metabolic activation in experiment II and in the untreated control in experiment I with metabolic activation. Since this deviation is rather small it is judged to be based on biologically irrelevant fluctuations in the number of colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, SYN545192 tech is considered to be non-mutagenic in this *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Materials and Methods

Study design and methods
Study experimentation dates - Start: March 04, 2011 End: August 29, 2011

Materials

Test material:

Description: No description provided
Lot/Batch #: SMU9BP005-FORTIFIED
Purity: 97.6 %
CAS #:
Stability in Solvent: Not indicated by the sponsor

Control materials:

Negative: Concurrent untreated and solvent controls were performed.

Positive: Non-activation:

- Sodium azide (NaN_3) 10 µg/plate TA1535, TA100

4-nitro-o-phenylene-diamine (4-NOPD) 10 µg/plate in TA 98; 50 µg/plate in TA 1537

- Methyl methane sulfonate (MMS) 3 µL/plate. WP2 *uvrA* (pKM101), WP2 (pKM101)

Activation:

- 2-Aminoanthracene (2-AA) 2.5 µg/plate (TA1535, TA1537, TA98, TA100); 10 µg/plate (WP2 *uvrA* pKM101, WP2 pKM101)

Activation:

S9 mix composition:

8mM $MgCl_2$
33 mM KCl
5 mM Glucose-6-phosphate
4 mM NADP

In 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

Test organisms:
Salmonella typhimurium strains: TA1535, TA1537, TA98, and TA100
Escherichia coli strains: WP2 *uvrA* pKM101 and WP2 pKM101

Test compound concentrations used:
Preliminary Test:
To evaluate the toxicity of the test item a pre-experiment was performed with strains TA 1535, TA 1537, TA 98, TA 100, WP2 uvrA pKM 101, and WP2 pKM 101. Eight concentrations were tested for toxicity and mutation induction each with three replicate plates.

Main Assay (all strains):

Pre-Experiment/Experiment I:
3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II:
3; 10**;** 33; 100; 333; 1000; 2500; and 5000 µg/plate

Test performance

Protocol. In each experiment, for each strain and dose level (including controls) three plates were used.
The following materials were mixed in a test tube and poured onto the selective agar plates:
100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 µL S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µL Bacteria suspension (test system, pre-culture of the strains)
2000 µL Overlay agar

For the pre-incubation method 100 µL test solution, 500 µL S9 mix / S9 mix substitution buffer (7 parts of 100 Mm Sodium-ortho-phosphate buffer pH 7.4 with 3 parts of KCl solution 0.15 M) and 100 µL bacteria suspension were mixed in a test tube and incubated at 37 °C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on selective agar plates.
After solidification the plates were incubated upside down for 48 - 72 hours at 37°C in the dark.

Appropriate reference mutagens were used as positive controls, which showed a distinct increase of induced revertant colonies.

Statistics – According to the OECD guideline 471, a statistical analysis of the data is not mandatory.

Results

Preliminary assay – In the pre-experiment the concentration range of the test item was 3 - 5000 µg /plate. The pre-experiment was reported as main experiment. Based on the minor toxic effects observed the same concentration range was tested and 5000 µg /plate was chosen as maximum concentration for experiment II.

Mutagenicity assay –The plates incubated with the test item showed normal background growth up to 5000 µg /plate with and without metabolic activation in both independent experiments.
Minor toxic effects were observed in strain TA 1537 at 2500 and 5000 µg /plate with metabolic activation in experiment I. No toxic effects were observed in the remaining strains and in experiment II.
Precipitation of the test item was observed in the overlay agar in the test tubes and on the incubated agar plates from 1000 - 5000 µg /plate in both experiments. The undissolved particles had no influence on the data recording.
No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with SYN546482 at any dose level, neither in the presence nor absence of metabolic activation (S9

mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.
The laboratory´s historical control range was slightly exceeded in the solvent control of strain WP2 pKM101 with and without metabolic activation in experiment I, and without metabolic activation in experiment II and in the untreated control in experiment I with metabolic activation. Since this deviation is rather small it is judged to be based on biologically irrelevant fluctuations in the number of colonies.

Table 1. Number of Revertant Colonies (group mean & standard deviation), without activation test
Experiment I

Without Activation						
	Revertant Colony Counts (Mean ±SD)					
Dose (µg/plate)	TA1535	TA1537	TA 98	TA100	WP2 pKM101	WP2 uvrA pKM101
DMSO	15 ± 6	14 ± 5	32 ± 7	124 ± 12	275 ± 10	460 ± 15
CONTROL	13 ± 1	11 ± 5	39 ± 5	122 ± 7	271 ± 15	434 ± 39
3	16 ± 4	14 ± 7	26 ± 3	129 ± 14	261 ± 17	475 ± 29
10	15 ± 5	19 ± 2	30 ± 4	129 ± 17	275 ± 11	473 ± 15
33	15 ± 4	15 ± 5	30 ± 2	136 ± 12	270 ± 11	444 ± 20
100	17 ± 2	13 ± 5	29 ± 6	114 ± 6	279 ± 7	452 ± 29
333	16 ± 5	12 ± 4	38 ± 5	111 ± 12	249 ± 21	445 ± 22
1000	16 ± 1 [P]	12 ± 2 [P]	20 ± 2 [P]	101 ± 9 [P]	270 ± 7 [P]	417 ± 14 [P]
2500	11 ± 1 [PM]	10 ± 1 [P]	17 ± 2 [PM]	88 ± 8 [PM]	200 ± 11 [PM]	345 ± 8 [PM]
5000	12 ± 3 [PM]	11 ± 3 [PM]	10 ± 2 [PM]	70 ± 5 [PM]	101 ± 8 [PM]	217 ± 12 [PM]
Positive						
NaN3 10 µg	1858 ± 68			2042 ± 55		
4-NOPD 10 µg			353 ± 36			
4-NOPD 50 µg		91 ± 15				
MMS 3.0 µL					3596 ± 152	3588 ± 63
With Activation						

Dose (µg/plate)	TA1535	TA1537	TA 98	TA100	WP2 pKM101	WP2 uvrA pKM101
DMSO	19 ± 3	19 ± 4	39 ± 10	148 ± 20	312 ± 14	496 ± 10
CONTROL	18 ± 3	24 ± 7	42 ± 8	146 ± 11	313 ± 10	485 ± 18
3	17 ± 3	21 ± 5	38 ± 8	132 ± 5	301 ± 20	473 ± 12
10	21 ± 5	17 ± 6	34 ± 4	152 ± 21	295 ± 29	485 ± 19
33	20 ± 5	17 ± 3	36 ± 6	166 ± 17	253 ± 5	469 ± 13
100	18 ± 3	23 ± 1	39 ± 4	151 ± 15	275 ± 41	408 ± 27
333	22 ± 1	21 ± 2	49 ± 8	127 ± 2	281 ± 4	471 ± 13
1000	21 ± 5 P	22 ± 11 P	40 ± 5 P	107 ± 12 P	272 ± 17 P	431 ± 60 P
2500	18 ± 4 PM	12 ± 3 PM	20 ± 2 PM	89 ± 4 PM	167 ± 8 PM	392 ± 14 PM
5000	10 ± 1 PM	10 ± 3 PM	17 ± 3 PM	66 ± 4 PM	133 ± 11 PM	370 ± 4 PM
Positive						
2-AA 2.5 µg	423 ± 17	421 ± 33	2514 ± 292	2612 ± 134		
2-AA 10 µg					1745 ± 111	2244 ± 88

Table 2. Number of Revertant Colonies (group mean / standard deviation), test Experiment II

Without Activation						
	Revertant Colony Counts (Mean ±SD)					
Dose (µg/plate)	TA1535	TA1537	TA 98	TA100	WP2 pKM101	WP2 uvrA pKM101
DMSO	17 ± 4	15 ± 1	34 ± 2	127 ± 13	238 ± 2	397 ± 6
CONTROL	15 ± 2	12 ± 2	36 ± 2	158 ± 13	268 ± 8	417 ± 17
3	18 ± 6	16 ± 3	35 ± 2	125 ± 8	247 ± 12	389 ± 18
10	15 ± 6	15 ± 5	35 ± 5	120 ± 7	254 ± 15	388 ± 12
33	19 ± 9	14 ± 8	37 ± 8	130 ± 5	240 ± 15	395 ± 9
100	21 ± 2	11 ± 3	30 ± 6	117 ± 9	252 ± 14	369 ± 22
333	13 ± 4	9 ± 1	28 ± 3	90 ± 13	231 ± 3	382 ± 11

1000	15 ± 2 P	10 ± 2 P	35 ± 6 P	93 ± 12 P	254 ± 13 P	371 ± 60 P
2500	15 ± 3 P	6 ± 3 PM	18 ± 3 PM	89 ± 6 PM	245 ± 25 PM	391 ± 23 PM
5000	12 ± 4 PM	7 ± 3 PM	23 ± 5 PM	66 ± 9 PM	206 ± 29 PM	368 ± 5 PM
Positive						
NaN3 10 µg	1906 ± 42			2017 ± 70		
4-NOPD 10 µg			365 ± 13			
4-NOPD 50 µg		100 ± 17				
MMS 3.0 µL					3087 ± 349	2595 ± 84

With Activation						
	Revertant Colony Counts (Mean ±SD)					
Dose (µg/plate)	TA1535	TA1537	TA 98	TA100	WP2 pKM101	WP2 *uvr*A pKM101
DMSO	15 ± 2 BM	17 ± 2	45 ± 5	156 ± 13	250 ± 33	452 ± 27
CONTROL	14 ± 2 BM	15 ± 1	44 ± 3	190 ± 3	316 ± 15	462 ± 11
3	17 ± 4 BM	16 ± 1	40 ± 5	147 ± 12	278 ± 5	441 ± 23
10	16 ± 4 BM	14 ± 2	38 ± 7	152 ± 7	244 ± 23	416 ± 10
33	17 ± 1 BM	15 ± 5	46 ± 3	157 ± 9	226 ± 16	404 ± 8
100	17 ± 2 BM	12 ± 2	47 ± 8	162 ± 4	229 ± 9	380 ± 4
333	16 ± 3 BM	14 ± 2	69 ± 10	132 ± 5	278 ± 19	404 ± 45
1000	16 ± 2 PBM	15 ± 4 P	58 ± 6 P	123 ± 6 P	273 ± 12 P	346 ± 18 P
2500	16 ± 1 PMB	8 ± 2 PM	33 ± 4 PM	126 ± 13 PM	231 ± 18 PM	353 ± 8 PM
5000	15 ± 2 PMB	10 ± 2 PM	32 ± 5 PM	105 ± 4 PM	219 ± 15 PM	330 ± 32 PM
Positive						
2-AA 2.5 µg	337 ± 9 BM	282 ± 10	2003 ± 109	2245 ± 62		
2-AA 10 µg					1538 ± 222	2342 ± 69

Discussion

Investigators' conclusions – "During the described mutagenicity tests and under the experimental conditions reported, SYN545192 tech. did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. SYN545192 tech. is considered to be non-mutagenic in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay."

Reviewer comments – The reviewer agrees with the study author's conclusion. There was no evidence of induced mutant colonies over background. SYN545192 tech did not induce mutagenicity in any of the bacterial tester strains in either the presence or absence of metabolic activation according to the described methods.
This reviewer noted the minor toxic effects observed in strains TA 1537, TA98, TA100, WP2pKM101 and WP2 uvrApKM101 at 2500 and 5000 µg /plate with metabolic activation in experiment I and considers that these minor effects had no detrimental impact on the outcome of the study.

We noticed the occurrence of precipitation in the experiment I, from the concentration of 333 µg/plate to 5000 µg/plate, and in experiment II from µg/plate 1000 to 5000 µg/plate. It would be important to clarify why did the samples precipitate in the experiments I and II from the concentrations mentioned above.

Study deficiencies – This reviewer found no deficiencies

B.6.4.2 Study Type: SYN545192-Chromosome Aberration Test in Human Lymphocytes *in Vitro*. OECD 473 (1997), EPA OPPTS 870.5375 (1998), EC440/2008 B.10 (2008).

Test Material SYN545192

(purity): 97%

Batch No.: SMU9BP005

Molecular Weight: 398.2 g/mol

Colour: beige powder

Citation: Report Number: 1258903. Study report date: May 17, 2010.

Sponsor: Syngenta Crop Protection, LLC, Greensboro, NC 27419-8300 USA.

Study Number: 1258903

Executive Summary:
This *in vitro* assay was performed to assess the potential of SYN545192 to induce structural chromosomal aberrations in the absence and the presence of an exogenous metabolic activation system (liver S9 mix from phenobarbital/_-naphthoflavone treated male rats).

In each experimental group two parallel cultures were analysed. Per culture 100 metaphase plates were scored for structural chromosomal aberrations.

In all experimental parts cytotoxicity was observed at the highest evaluated concentration.

In both independent experiments, no biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment with the test item.

No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.

Appropriate mutagens were used as positive controls. They induced statistically significant increases ($p < 0.05$) in cells with structural chromosome aberrations

In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce structural chromosomal aberrations in human lymphocytes in vitro. SYN545192 is considered to be non-clastogenic in this chromosome aberration test in the absence and presence of metabolic activation when tested up to cytotoxic concentrations.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Materials and Methods

Materials

1. Test material:

Description: SYN545192
Lot/Batch #: SMU9BP005
Molecular Weight: 398.2 g/mol
Colour: beige powder
Purity: 97 %
Solvent used: Not indicated by the Sponsor

Control materials:
Negative: 0.5 % (v/v) **DMSO in culture medium**
Positive: Non-activation: **Ethyl methanesulfonate (EMS)**
Final concentration: 825.0 µg/mL (Exp. I)
770.0 µg/mL (Exp. II)
Activation: **Cyclophosphamide (CPP)**
Final concentration: 30.0 µg/mL (Exp. I)
15.0 µg/mL (Exp. II)

Activation: S9 derived from

X	Induced		Aroclor 1254	**X**	Rat	**X**	Liver
	Non-induced	**X**	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	ß-naphthoflavone		Other		

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/mL in the cultures. Cofactors were added to the S9 mix to reach the following concentrations:

8 mM MgCl2
33 mM KCl
5 mM glucose-6-phosphate
4 mM NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.
During the experiment, the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames et al. (1).

Test cells: Blood samples were obtained from healthy donors not receiving medication. For this study, blood was collected from a female donor (35 years old) for the first experiment and from a 47 year-old female donor for Experiment II.
Blood samples were drawn by venous puncture and collected in heparinized. The tubes were sent to Harlan CCR to initiate cell cultures within 24 hrs after blood collection. If necessary, the blood was stored before use at 4 °C. Blood cultures were set up in bulk within 24 hrs after collection in 75 cm² cell culture flasks (Greiner, 72632 Frickenhausen, Germany). The culture medium was DMEM:F12 (Dulbecco's modified eagle medium/ Ham's F12 medium; mixture 1:1; Life Technologies GmbH, 76339 Eggenstein, Germany) containing 10 % FCS (fetal calf serum) provided by PAA Laboratories GmbH (35091 Cölbe, Germany). The antibiotic solution contains 10,000 U/mL penicillin and 10,000 µg/mL streptomycin (SEROMED, 12247 Berlin,Germany). Additionally, the medium was supplemented with Phytohemagglutinin (PHA, final concentration 3 µg/mL, SEROMED), the anticoagulant heparin (25,000 U.S.P.-U/mL, NATTERMANN, 50829 Köln, Germany), and HEPES (final concentration 10 mM, Serva, 69115 Heidelberg, Germany).

The following volumes are added to the flasks (per 10 mL):
7.70 mL culture medium
1.00 mL fetal calf serum
0.10 mL L-glutamine
0.10 mL antibiotic solution
0.10 mL HEPES
0.05 mL phytohemagglutinin
0.05 mL heparin
0.90 mL whole blood
All incubations were done at 37 °C in a humidified atmosphere with 5.5 % CO2 (94.5 % air).

Test compound concentrations used (µg/mL):

TABLE 1. Doses Applied in the Chromosome Aberration Assay with SYN545192

Exp	Prep.interva	Expos. Period.	Concentrations in µg/mL											
			Without S9 mix											
I	22 hrs	4 hrs	3.1*	5.5*	9.6*	16.9	29.5	51.7 P	90.5 P	158.3 P	277.1 P	484.9 P	848.6 P	1485.0 P
II	22 hrs	22 hrs	0.06	0.11	0.19	0.34*	0.6*	1.04	1.83*	3.2*	5.6	9.8	17.14	30
			With S9 mix											
I	22 hrs	4 hrs	3.1*	5.5*	9.6*	16.9	29.5 P	51.7 P	90.5 P	158.3 P	277.1 P	484.9 P	848.6 P	1485.0 P
II	22 hrs	4 hrs					1.3	2.5*	5*	7.5*	10*	15	20	30

* Evaluated experimental points

P Precipitation was observed at the end of treatment

Test performance

Preliminary cytotoxicity assay –Using reduced mitotic indices as an indicator for toxicity in Experiment I, toxic effects were observed after 4 hrs treatment in the absence and presence of S9 mix. Considering the toxicity data of Experiment I, 30.0 µg/mL (with and without S9 mix) was chosen as top concentration in Experiment II. The applied concentrations in the cytogenetic experiments are presented in Table 1.

Experimental performance cytogenetic experiment
Two independent experiments were performed. In Experiment I, the exposure period was 4 hours with and without S9 mix. In Experiment II, the exposure periods were 4 hours with S9 mix and 22 hours without S9 mix. The chromosomes were prepared 22 hours after start of treatment with the test item.

Schedule

	Without S9 mix		With S9 mix	
	Exp. I	Exp. II	Exp. I	Exp. II
Exposure period	4 hrs	22 hrs	4 hrs	4 hrs
Recovery	18 hrs		18 hrs	18 hrs
Preparation interval	22 hrs	22 hrs	22 hrs	22 hrs

Treatment
Exposure time 4 hours
About 70 hrs after seeding for each test group 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks (Nunc GmbH & Co. KG, 65203 Wiesbaden, Germany). The culture medium was replaced with

serum-free medium containing the test item. For the treatment with metabolic activation 50 LL S9 mix per mL medium were used. Concurrent solvent and positive controls were performed. After 4 hrs the cells were spun down by gentle centrifugation for 5 minutes. The supernatant with the dissolved test item was discarded and the cells were re-suspended in "saline G". The washing procedure was repeated once as described.
The "saline G" solution was composed as follows (per litre):
NaCl 8000 mg
KCl 400 mg
Glucose × H_2O 1100 mg
Na_2HPO_4 × $7H_2O$ 290 mg
KH_2PO_4 150 mg
pH was adjusted to 7.2
After washing the cells were re-suspended in complete culture medium and cultured until preparation.

Exposure time 22 hours (without S9 mix)
About 70 hrs after seeding for each test group 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks (Nunc GmbH & Co. KG, 65203 Wiesbaden, Germany). The culture medium was replaced with complete medium (with 10 % FCS) containing the test item without S9 mix. The culture medium at continuous treatment was not changed until preparation of the cells. Concurrent solvent and positive controls were performed. All cultures were incubated at 37 °C in a humidified atmosphere with 5.5 % CO2 (94.5 % air).

Preparation of the cultures.
Three hours before harvesting, colcemid (Fluka, 89203 Neu-Ulm, Germany) was added to the cultures (final concentration 0.2 Lg/mL). The cultures were harvested by centrifugation 22 hrs after beginning of treatment. The supernatant was discarded and the cells were resuspended in approximately 5 mL hypotonic solution (0.0375 M KCl). The cell suspension was then allowed to stand at 37 °C for 20 to 25 minutes. After removal of the hypotonic solution by centrifugation the cells were fixed with a mixture of methanol and glacial acetic acid (3 parts plus 1 part). At least two slides per experimental group were prepared by dropping the cell suspension onto a clean microscope slide. The cells for evaluation of cytogenetic damage were stained with Giemsa (MERCK, 64293 Darmstadt, Germany).

Metaphase analysis.
Gaps were recorded as well, but they were not included in the calculation of the aberration rates. 100 well spread metaphase plates per culture were scored for cytogenetic damage on coded slides. Only metaphases with 46 ± 1 centromer regions were included in the analysis. To describe a cytotoxic effect the mitotic index (% cells in mitosis) was determined.

Evaluation criteria
A test item is classified as non-mutagenic if:
-the number of induced structural chromosome aberrations in all evaluated dose groups is in the range of the historical control data.
- No significant increase of the number of structural chromosome aberrations is observed.

A test item is classified as mutagenic if:
- The number of induced structural chromosome aberrations is not in the range of the historical control data.
- Either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed.

Statistics - Statistical significance was confirmed by means of the Fisher´s exact test (10) ($p < 0.05$). However, both biological and statistical significance should be considered together. If the above mentioned criteria for the

test item are not clearly met, the classification with regard to the historical data and the biological relevance is discussed and/or a confirmatory experiment is performed.

Reported Results

Preliminary cytotoxicity assay – In Experiment I, in the absence of S9 mix, precipitation of the test item in the culture medium was observed with 51.7 µg/mL and above and with 29.5 µg/mL and above in the presence of S9 mix.
No relevant increase in the osmolarity or pH value was observed (e.g. Exp. I: solvent control: 375 mOsm, pH 7.3 versus 373 mOsm and pH 7.4 at 1485.0 µg/mL).In all experimental parts cytotoxicity was observed at the highest evaluated concentration.
In detail, in Experiment I, the mitotic index was reduced to 38.7 % (without S9 mix) and 47.8 % (with S9 mix). In Experiment II, a reduction to 40.7 % (without S9 mix) and 39.1 % (with S9 mix) was observed.

Table 2. Experiment I (Cytotoxicity of SYN545192 to the Cultures of Human Lymphocytes). In Experiment I the mitotic index in two cultures (1000 cells per culture) was determined.

Concentration (µg/mL)	Exposure time	Preparation interval	Mitotic cells per 1000 cells*	% of solvent control
Without S9 mix				
Solvent control	4 hrs	22 hrs	14.1	100
3.1	4 hrs	22 hrs	9.6	68.1
5.5	4 hrs	22 hrs	7.1	50
9.6	4 hrs	22 hrs	5.5	38.7
16.9	4 hrs	22 hrs	4.8	34
29.5	4 hrs	22 hrs	5	35.1
51.7 P	4 hrs	22 hrs	3.6	25.2
90.5 P	4 hrs	22 hrs	0.0	0.0
158.3 P	4 hrs	22 hrs	0.0	0.0
277.1 P	4 hrs	22 hrs	0.0	0.0
484.9 P	4 hrs	22 hrs	0.0	0.0
848.6 P	4 hrs	22 hrs	0.0	0.0
1485.0 P	4 hrs	22 hrs	0.0	0.0

Experimental groups evaluated for cytogenetic damage are shown in bold characters

* Mean value of two cultures in %

P Precipitation was observed at the end of treatment

Table 3. Experiment I (Cytotoxicity of SYN545192 to the Cultures of Human Lymphocytes) (continued). In Experiment I the mitotic index in two cultures (1000 cells per culture) was determined.

Concentration (µg/mL)	Exposure time	Preparation interval	Mitotic cells per 1000 cells*	% of solvent control
With S9 mix				
Solvent control	4 hrs	22 hrs	10.1	100
3.1	4 hrs	22 hrs	10.4	103.5
5.5	4 hrs	22 hrs	6.2	61.2
9.6	4 hrs	22 hrs	4.8	47.8
16.9	4 hrs	22 hrs	3.8	37.3
29.5	4 hrs	22 hrs	3.4	33.3
51.7 P	4 hrs	22 hrs	3.8	37.3
90.5 P	4 hrs	22 hrs	3.3	32.3
158.3 P	4 hrs	22 hrs	1.4	13.9
277.1 P	4 hrs	22 hrs	0.0	0.0
484.9 P	4 hrs	22 hrs	0.0	0.0
848.6 P	4 hrs	22 hrs	0.0	0.0
1485.0 P	4 hrs	22 hrs	0.0	0.0

Experimental groups evaluated for cytogenetic damage are shown in bold characters

* Mean value of two cultures in %

P Precipitation was observed at the end of treatment

Cytogenetic assay – In both experiments, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. The aberration rates of the cells after treatment with the test item (0.5 – 3.5 % aberrant cells, excluding gaps) exceeded the solvent control values (1.0 – 1.5 % aberrant cells, excluding gaps) but were within the range of the laboratory's historical solvent control data (0.0 – 4.0 % aberrant cells, excluding gaps).

No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.

In both experiments, either EMS (770 or 825 µg/mL) or CPA (15 or 30 µg/mL) were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

Table 2. Experiment I: Summary of Results

	Activated with S9				
Dose (µg/mL)	Cell Number (% control)	Mitotic Index (% control)	Polyploid Cells (%)	Aberrant cells (%) Excl. Gaps	Aberrant cells (%) Incl. Gaps
4-hr exposure with 18 hour sampling time					
Vehicle Control	100	100	0.5	1.0	2.5
10	101.7	nd	nd	nd	nd
20	99.0	nd	nd	nd	nd
40	91.6	107.6	0.0	4.0	7.5
80	71.9	76.3	0.5	1.5	3.5
120*	66.2	61.6	0.0	4.5	7.5
160*	34.8	11.2	ns	ns	ns
Positive Control	nt	80.4	0.0	22.0	24.0

nt = not tested
nd = not determined
ns = not scoreable due to poor metaphase quality and/or strong cytotoxicity
* Precipitation of test article

Table 3. Experiment II: Summary of Results

	Not activated with S9				
Dose (µg/mL)	Cell Number (% control)	Mitotic Index (% control)	Polyploid Cells (%)	Aberrant cells (%) Excl. Gaps	Aberrant cells (%) Incl. Gaps
4-hr exposure with 18 hour sampling time					
Vehicle Control	100	100	1.0	1.5	4.5
0.31	104.7	145.1	0.0	1.5	2.0
0.63	64.8	87.3	0.5	1.5	1.5
1.25	49.5	94.8	0.0	1.5	1.5
2.50	52.8	90.2	ns	ns	ns
5.00	61.1	ns	ns	ns	ns
10.00	35.5	ns	ns	ns	ns
Positive Control	nt	85.0	0.0	13.0	13.0

nt = not tested
nd = not determined
ns = not scoreable due to poor metaphase quality and/or strong cytotoxicity

Table 4. Experiment III: Summary of Results

	Not activated with S9				
Dose (µg/mL)	Cell Number (% control)	Mitotic Index (% control)	Polyploid Cells (%)	Aberrant cells (%) Excl. Gaps	Aberrant cells (%) Incl. Gaps
18-hr exposure with 18 hour sampling time					
Vehicle Control	100	100	0.0	1.0	4.0
0.31	99.8	nd	nd	nd	nd
0.63	104.4	nd	nd	nd	nd
1.25	94.3	nd	nd	nd	nd
2.50	88.7	156.6	0.0	2.0	4.5
5.00	102.0	144.1	1.0	3.0	5.0
10.00	85.0	104.4	0.0	2.5	3.5
20.00	61.8	ns	ns	ns	ns
40.00	54.4	ns	ns	ns	ns
Positive Control	nt	113.2	0.0	28.0	32.0

nt = not tested
nd = not determined
ns = not scoreable due to poor metaphase quality and/or strong cytotoxicity

Table 5. Experiment III: Summary of Results

	Not activated with S9				
Dose (µg/mL)	Cell Number (% control)	Mitotic Index (% control)	Polyploid Cells (%)	Aberrant cells (%) Excl. Gaps	Aberrant cells (%) Incl. Gaps
18-hr exposure with 28 hour sampling time					
Vehicle Control	100	100	0.0	2.5	6.5
2.50	99.2	nd	nd	nd	nd
5.00	109.3	nd	nd	nd	nd
10.00	87.0	87.8	1.5	1.5	2.0
20.00	78.1	6.8	ns	ns	ns
40.00	5.3	ns	ns	ns	ns
Positive Control	nt	76.8	0.0	39.0	39.0

nt = not tested
nd = not determined
ns = not scoreable due to poor metaphase quality and/or strong cytotoxicity

Table 6: Experiment III: Summary of Results

	Activated with S9				
Dose (µg/mL)	Cell Number (% control)	Mitotic Index (% control)	Polyploid Cells (%)	Aberrant cells (%) Excl. Gaps	Aberrant cells (%) Incl. Gaps
4-hr exposure with 18 hour sampling time					
Vehicle Control	100	100	0.0	2.0	4.5
20	94.3	nd	nd	nd	nd
40	118.0	77.9	0.5	2.5	7.0
80	128.3	86.3	0.0	5.0	8.0
120*	105.7	99.2	0.0	1.0	2.0
160*	150.4	ns	ns	ns	ns
240*	153.7	ns	ns	ns	ns
320*	163.1	ns	ns	ns	ns
Positive Control	nt	104.2	2.0	23.0	27.0

nt = not tested
nd = not determined
ns = not scoreable due to poor metaphase quality and/or strong cytotoxicity
* Precipitation of test article

Discussion

Investigators' conclusions –"In conclusion, it can be stated that under the experimental conditions reported, the test item SYN545192 did not induce structural chromosomal aberrations in human lymphocytes *in vitro* in the absence and presence of metabolic activation".

Reviewer comments –There was no evidence of chromosome aberration induced over background.

Study deficiencies – None.

Study type: SYN545192 – Cell Mutation Assay at the Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y Cells. Wollny H-E., Germany. Laboratory report number: 1258902. Study report date: 06-September-2010.

Test Material (purity): 97 %

Synonyms: SYN545192

Sponsor: Syngenta Crop Protection, Inc. Greensboro, USA.

Executive Summary: In a mammalian cell gene mutation assay at the thymidine kinase (TK) locus, L5178Y mouse lymphoma cells cultured *in vitro* were exposed to SYN545192 (97%) in DMSO.
The assay was performed in three independent experiments, using two parallel cultures each. The main experiments were evaluated with the following concentrations: Experiment I (without S9 mix): 2.5, 5.0, 10.0, 15.0, 20.0 µg/mL (and with S9 mix): 5.0, 10.0, 20.0, 30.0, 40.0 µg/mL, experiment II (without S9 mix): 5.0, 10.0, 20.0, 30.0 µg/mL, (with S9 mix): 10.0, 20.0, 30.0, 40.0, 50.0 µg/mL, and experiment III: (without S9 mix) 2.5, 5.0, 10.0, 20.0, 30.0 µg/mL

The first two main experiments were performed with and without liver microsomal activation and a treatment period of 4 hours. The third experiment was solely performed without metabolic activation and a treatment time of 4 hours. The purpose of the third experiment was to cover the cytotoxic range that was not reached in the first experiment without metabolic activation.

The highest applied concentration (1500 µg /mL in the range-finding pre-experiment) was chosen with regard to the solubility properties of the test item in organic solvents and aqueous medium. The concentration range of the main experiments was limited to 60 µg /mL by cytotoxic effects.
No substantial and reproducible dose dependent increase in mutant colony numbers was observed in both main experiments. No relevant shift of the ratio of small versus large colonies was observed up to the maximum concentration of the test item.

In conclusion it can be stated that during the mutagenicity test described and under the experimental conditions reported SYN545192 did not induce mutations with and without metabolic activation.

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. Materials and Methods

A. Materials

1. Test material: SYN545192
Description: Beige powder
Lot/Batch #: SMU9BP005
Purity: 97 %
CAS #:
Solvent used: DMSO

2. Control materials:
Negative: DMSO
Positive: Non-activation: Methyl methane sulfonate (MMS)
Activation: Cyclophosphamide (CPA)

3. Activation: S9 derived from

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	ß-naphthoflavone		Other		

S9 mix composition

The protein concentration of the S9 preparation was 35.0 mg in the pre-experiment. In experiments I, and II the protein concentration was 35.6 mg/mL

8 mM $MgCl_2$
33 mM KCl
5 mM glucose-6-phosphate
4 mM NADP

In 100 mM sodium-ortho-phosphate-buffer, pH 7.4.
The concentration in the final test medium was 5 % (v/v).

4. Test cells: mammalian cells in culture

X	mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells		list any others

Medium: RPMI 1640-HAP

Prior to mutagenicity testing the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with: hypoxanthine (1.0×10^{-4} M), aminopterin (2.0×10^{-7} M) and thymidine (1.6×10^{-5} M). The incubation of the cells in HAT-medium was followed by a recovery period of 2 days in RPMI 1640 medium containing: hypoxanthine (1.0×10-4 M) and thymidine (1.6×10-5 M).

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No

5. **Locus Examined:** X Thymidine kinase (TK) Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) Na^+/K^+ ATPase

Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		fluorodeoxyuridine (FdU)		6-thioguanine (6-TG)		
	X	trifluorothymidine (TFT)				

6. **Test compound concentrations used**:

The main experiments were evaluated at the following concentrations:

Experiment I:

without S9 mix: 2.5, 5.0, 10.0, 15.0, 20.0 µg/mL
with S9 mix: 5.0, 10.0, 20.0, 30.0, 40.0 µg/mL

Experiment II:

- without S9 mix: 5.0, 10.0, 20.0, 30.0 µg/mL
- with S9 mix: 10.0, 20.0, 30.0, 40.0, 50.0 µg/mL

Experiment III:

- without S9 mix: 2.5, 5.0, 10.0, 20.0, 30.0 µg/mL

B. Test performance

1. Cell treatment -

a. 1 x 10^7 cells were exposed to test compound, negative/solvent or positive control substance for 4 hours (non-activated and activated).

b. After washing, cells were cultured for 2 days (expression and growth period) before cell selection.

c. The cell density was determined each day and adjusted to 3×10^5 cells/mL, if necessary.

d. After expression, the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4×10^3 cells in selective medium with TFT. The viability (cloning efficiency 2) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37°± 1.5 °C in 4.5 % CO2/95.5 % water saturated air for 10 - 15 days. Then the plates were evaluated.

Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the large colonies). The survival rate and viability were determined based on the Poisson distribution method. The zero term of the Poisson distribution, [P(0)] method, was used. The mutation frequency was derived from the cloning efficiency under selective conditions compared to the corresponding viability under non-selective conditions.

2. Statistics – A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT_11 statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend was judged as significant whenever the p-value (probability value) was below 0.05. Both, biological relevance and statistical significance were considered together.

3. Evaluation criteria

- A test item was classified as mutagenic if the induced mutation frequency reproducibly exceeded a threshold of 126 colonies per 106 cells above the corresponding solvent control or negative control, respectively.
- A relevant increase of the mutation frequency should be dose-dependent.
- A mutagenic response was considered to be reproducible if it occurred in both parallel cultures.
- In the evaluation of the test results the historical variability of the mutation rates in negative and vehicle controls and the mutation rates of all negative and vehicle controls were taken into consideration.
- Results of test groups were generally rejected if the relative total growth and the cloning efficiency 1 is less than 10 % of the vehicle control unless the exception criteria specified by the IWGT recommendations were fulfilled.
- Whenever a test item was considered mutagenic, the ratio of small versus large colonies was used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency was accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated.

II. Reported Results

A. <u>Pre-experiment</u>– A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. Both pH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation. 1×10^7 cells were exposed to each concentration of the test item for 4 hours with and without metabolic activation. Following treatment the cells were washed twice by centrifugation (425 g, 10 min) and resuspended in "saline G". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to 3×10^5 cells/mL, if necessary.

Table 1. Cell count, pre-experiment

	Conc. µg per mL	S9 mix	Number of cells per mL (4h after treatment)	Number of cells per mL (24h after treatment)	Number of cells per mL (48h after treatment)	Total suspension growth TSG	Relative suspension growth RSG
Solvent control w/ DMSO		-	446000	1047600	1514200	11.9	100.0
Test item	11.7	-	378000	544800	1013800	4.9	41.1
Test item	23.4	-	89600	73200	61000	0.7	5.7
Test item	46.9	-	168800	75800	46600	0.3	2.3
Test item	93.8	-	185200	61000	42600	0.2	1.9
Test item	187.5	-	182800	43200	nd	nd	nd
Test item	375.0	-	141000	37000	nd	nd	nd
Test item	750.0	-	139000	30800	nd	nd	nd
Test item	1500.0	-	160600	59200	nd	nd	nd
Solvent control w/ DMSO		+	478600	594400	1547200	6.4	100.0
Test item	11.7	+	496400	368600	1590000	3.9	61.4
Test item	23.4	+	390600	241600	582200	1.5	23.3
Test item	46.9	+	141000	43400	79200	0.6	8.8
Test item	93.8	+	162200	38000	60000	0.4	5.8
Test item	187.5	+	138000	nd	nd	nd	nd
Test item	375.0	+	110200	nd	nd	nd	nd
Test item	750.0	+	157200	nd	nd	nd	nd
Test item	1500.0	+	212600	nd	nd	nd	nd

nd: not determined due to exceedingly severe cytotoxic effects

B. <u>Mutagenicity assay</u> –

Table 2. Summary of Results. Experiment I

	Conc. µg per mL	S9 mix	Relative cloning efficiency 1	Relative total growth	Mutant colonies 10^6 cells	threshold	Relative cloning efficiency 1	Relative total growth	Mutant colonies 10^6 cells	threshold
			Culture I				**Culture II**			
Solv. Control w/ DMSO		-	100.0	100.0	112	238	100	100	133	259
Pos. control w/MMS	19.5	-	89.0	57.0	266	238	82.3	67.8	299	259
Test item	1.3	-	101.5	Culture was not continued			97.0	Culture was not continued		
Test item	2.5	-	107.7	92.9	85	238	128.7	168.0	78	259
Test item	5.0	-	120.1	99.3	80	238	106.5	101.7	90	259
Test item	10.0	-	132.5	96.4	125	238	95.5	103.0	115	259
Test item	15.0	-	106.1	89.1	134	238	94.1	127.2	82	259
Test item	20.0	-	87.8	99.4	117	238	110.0	76.6	115	259
Solv. Control w/ DMSO		+	100.0	100.0	104	230	100.0	100.0	73	199
Pos. control w/ CPA	3.0	+	46.8	33.8	90	230	79.6	67.6	250	199
Pos. control w/ CPA	4.5	+	4.9	2.2	472	230	8.2	7.6	355	199
Test item	2.5	+	101.8	Culture was not continued			86.7	Culture was not continued		
Test item	5.0	+	103.6	78.1	84	230	97.2	93.5	101	199
Test item	10.0	+	111.6	127.3	58	230	131.0	109.1	91	199
Test item	20.0	+	100.0	76.6	109	230	121.2	83.3	107	199
Test item	30.0	+	93.4	36.4	90	230	80.8	59.8	55	199

Test item	40.0	+	90.4	28.9	82	230	83.1	16.3	44	199

Table 3. Summary of results. Experiment II

	Conc. µg per mL	S9 mix	Relative cloning efficiency 1	Relative total growth	Mutant colonies 10^6 cells	threshold	Relative cloning efficiency 1	Relative total growth	Mutant colonies 10^6 cells	threshold
			Culture I				**Culture II**			
Solv. Control w/ DMSO		-	100.0	100.0	59	185	100	100	67	193
Pos. control w/MMS	19.5	-	109.3	53.6	431	185	74.0	43.0	352	193
Test item	2.5	-		Culture was not continued				Culture was not continued		
Test item	5.0	-	100.0	127	77	185	77.5	76.1	110	193
Test item	10.0	-	98.3	61.8	79	185	74.0	43.0	352	193
Test item	20.0	-	107.3	50.9	91	185	42.8	76.1	110	193
Test item	30.0	-	20.0	10.9	127	185	23.1	93.7	187	193
Test item	40.0	-	4.6	Culture was not continued			2.2	Culture was not continued		
Test item	50.0	-	4.6	Culture was not continued			0.3	Culture was not continued		
Test item	60.0	-	0.0	Culture was not continued			0.0	Culture was not continued		
Solv. Control w/ DMSO		+	100.0	100.0	74	200	100.0	100.0	86	212
Pos. control w/ CPA	3.0	+	79.2	55.4	245	200	66.1	47.7	275	212
Pos. control w/	4.5	+	42.8	26.1	525	200	41	34.2	379	212

CPA										
Test item	10.0	+	170.9	107.9	105	200	76.3	101.8	83	212
Test item	20.0	+	89.1	119.0	69	200	66.1	69.6	66	212
Test item	30.0	+	97.2	101.9	74	200	65.2	48.5	71	212
Test item	40.0	+	59.5	28.7	65	200	50.6	32.5	49	212
Test item	50.0	+	18.7	10.9	82	200	15.1	9.2	70	212
Test item	60.0	+	0.6	Culture was not continued			0.8	Culture was not continued		

Table 4. Summary of results. Experiment III

	Conc. µg per mL	S9 mix	Relative cloning efficiency 1	Relative total growth	Mutant colonies 10^6 cells	threshold	Relative cloning efficiency 1	Relative total growth	Mutant colonies 10^6 cells	threshold
			Culture I				**Culture II**			
Solv. Control w/ DMSO		-	100.0	100.0	65	191	100	100	67	197
Pos. control w/MMS	19.5	-	55.7	46.5	206	191	61.3	30.2	466	197
Test item	2.5	-	63.6	111.8	64	191	71.8	79.3	114	197
Test item	5.0	-	97.9	94.7	71	191	67.8	69.9	107	197
Test item	10.0	-	47.6	90.2	87	191	82.2	65.8	144	197
Test item	20.0	-	50.4	11.1	76	191	60.4	72.4	148	197
Test item	30.0	-	30.9	36.4	80	191	35.9	28.5	130	197
Test item	40.0	-	1.4	Culture was not continued			5.8	Culture was not continued		

- Relevant toxic effects indicated by a relative cloning efficiency 1 (survival) or a relative total growth (RTG) of less than 50% in both cultures occurred in the second experiment at 30 µg /mL and above without metabolic activation and at 40 µg /mL and above with metabolic activation. In the third experiment toxic effects were noted at 30.0 µg /mL and above. The recommended cytotoxic range of approximately 10-20% RTG was covered with and without metabolic activation.

- No substantial and reproducible dose dependent increase of the mutation frequency exceeding the threshold of 126 colonies per 106 cells above the corresponding solvent or negative control was observed in the presence and absence of metabolic activation up to the maximum concentration.

- A significant trend of the mutation frequency indicated by a probability value of <0.05 was solely determined in the first culture of the second experiment without metabolic activation. Since this trend was not reproduced in the parallel culture and the mutation frequency did not reach or exceed the threshold described above, the isolated trend was judged as biologically irrelevant.

- In this study the range of the solvent controls was from 59 up to 133 mutant colonies per 106 cells; the range of the groups treated with the test item was from 44 up to 148 mutant colonies per 106 cells. The solvent controls met all of the acceptance criteria in at least one of the parallel cultures.

- The positive control response was outside of the historical control range in a single culture in experiment III, however this was not considered to affect the validity of the assay due to the strong mutagenic response in culture II.

III. Discussion

A. Investigators' conclusions – "In conclusion it can be stated that during the mutagenicity test described and under the experimental conditions reported SYN545192 did not induce mutations with and without metabolic activation, Therefore, SYN545192 is considered to be non-mutagenic in this mouse lymphoma assay".

B. Reviewer comments - The reviewer agrees with the study author's conclusion.

C. Study deficiencies – This reviewer found no deficiencies

Citation: **SYN545192 – Micronucleus Test in Bone Marrow Cells of Wistar (Han) Rats**. Laboratory report number: 30255. Study report date: 23-August-2011.

Sponsor: Syngenta Crop Protection, LLC, Greensboro.

Executive Summary: In a bone marrow micronucleus assay, 24 male and female Wistar (Han) rats were treated with SYN545192 following a 0 h + 24 h oral dosing and 48 h sampling regimen. A set of dose range-finder toxicity studies were conducted to establish a suitable dose range for the micronucleus experiment. Based on the findings of the toxicity studies, the maximum tolerated dose of 175 mg SYN545192/kg/day was selected for male rats and 75 mg SYN545192/kg/day was selected for the female rats.

In the micronucleus test, a group of Wistar (Han) rats were therefore dosed at 0 h and 24 h *via* the oral route (gavage) with the test item at concentrations of 175 mg/kg/day, 87.5 mg/kg/day or 43.8 mg/kg/day in males or 75 mg/kg/day in females. Bone marrow samples were taken 48 h after the initial 0 h dose. Two control groups of Wistar (Han) rats were dosed orally with either the vehicle, 1% Carboxymethyl cellulose (CMC) with 0.1% TWEEN 80 at 10 mL/kg/day, or the positive control agent, 50 mg cyclophosphamide/kg/day.

There were no signs of toxicity or animal deaths noted during the study. The positive control induced the appropriate response. There were no significant increases in the frequency of micronucleated polychromatic erythrocytes in bone marrow at any dose level.

It was concluded that SYN545192 did not induce micronuclei in bone marrow cells when tested to the maximum tolerated doses of 175 mg/kg/day in male and 75 mg/kg/day in female Wistar (Han) rats using a 0 h + 24 h oral dosing and 48 h sampling regimen.

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5395; OECD 474 for in vivo cytogenetic mutagenicity data.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. Materials and Methods

A. Materials

1. **Test material:** **SYN545192**

Description:	Beige powder
Lot/Batch #:	SMU9BP005
Purity:	97 %.
Solvent used:	1% Carboxymethyl cellulose (CMC) with 0.1% TWEEN 80

2. **Control materials:**

Negative control (vehicle):	1 % Carboxynethyl cellulose CMC with 0.1% TWEEN 80	**Final Volume:** 10 mL/kg	**Route:** gavage
Positive control :	Cyclophosphamide (CPH)	**Final Dose:** 10 mg/kg	**Route:** gavage

3. **A. Test animals:**

Species:	Rat
Strain:	Wistar (Han)
Age at study initiation:	6-7 weeks
Weight at study initiation:	Males: 182-212 g, Females: 147-181 g.
Source:	Charles River UK (Kent, England)
No. animals used	Toxicity tests I and II: 3 males, 3 females. Micronucleus test: 30 males, 15 females (24 per dose)
Properly maintained?	Yes

B. Test compound administration: Dose: 43.8 mg/kg/day, 87.5 mg/kg/day, 175 mg/kg/day and 75 mg/kg/day. **Via:** Oral (Gavage)

4. Test performance

A. Toxicity study: In the first phase of the toxicity study, groups of male and female Wistar (Han) rats received oral doses of SYN545192 at 75 mg/kg/day. Clinical signs were observed in the female group, these included: hunched posture, staggering, subdued behaviour, piloerection and laboured breathing.

In the second phase of the toxicity study, male rats received oral doses of SYN545192 at 175 mg/kg/day. Mild clinical signs of hunched posture, staggering and subdued behavior were observed. Females in the second phase received 100 mg/kg/day.

1. Treatment and sampling times:

a. Test compound:

Dosing:	X	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr		24 hr	X	48 hr		72 hr
Other:										

b. Negative and/or vehicle control:

Dosing:	X	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr		24 hr	X	48 hr		72 hr
Other:										

c. Positive control:

Dosing:	X	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr		24 hr	X	48 hr		72 hr
Other:										

2 Tissues and cells examined:

Bone marrow	femoral
No. of polychromatic erythrocytes (PCE) examined per animal:	2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	1000
Other (if other cell types examined, describe):	

3. Details of slide preparation – Rats were killed by CO_2 asphyxiation. One femur of each rat was quickly dissected out and freed of adherent tissue. A small hole was made in the neck of one femur and the marrow flushed, using a 2 mL syringe fitted with a sterile needle, into a centrifuge tube containing 3 mL of a 1:1 mixture of fetal calf serum and 0.8% trisodium citrate in Sorensen's buffer, pH 6.8 (Sorensen's buffer, pH 6.8 = 2.84 g Na_2HPO_4/L plus 2.72 g KH_2PO_4/L distilled water). Routine tissue culture antibiotics were included to prevent microbial growth. This mildly hypotonic treatment served to make the micronuclei clearly visible and to distinguish them from surrounding artefacts.

The tubes were centrifuged to pellet the cells. All but a few drops of supernatant fluid were discarded. The cells were then resuspended on a vortex mixer in this residual amount of supernatant liquid. Clean slides were assigned numbers corresponding to the tube numbers. A drop of the suspension was placed at one end of the slide and a smear made by drawing the top of a Pasteur pipette horizontally along the slide. Two slides were prepared from each tube/animal. The smear was left to air dry, fixed in methanol for *ca* 5 min and then immersed for 15 min in 15% Giemsa stain, prepared in tap water, to give optimum erythrocyte discrimination. The stained smears were rinsed in 2-3 changes of water and left to air dry. Permanent slide preparations were made by sealing coverslips onto the glass slides using DPX mounting medium.

4. Evaluation criteria –

The average micronucleus incidence in vehicle control dosed and untreated Wistar (Han) rats, was determined as 0.06±0.05%, a range of 0.00-0.15%. These historical data were used in the evaluation of response.

The test would be judged negative if no biologically relevant increases in the numbers of MN-PCE were observed, relative to the concurrent and established historical control frequencies for MN-PCE induction. No statistical analysis would be performed if the levels of MN-PCE induction fell within the determined historical control frequencies.

The test would be judged positive if an increase in the number of micronucleated polychromatic erythrocytes (MN-PCE) was obtained for one or more of the test item treated dose groups. That is, an increase greater than 10% over the expected historical control ranges for a group of animals. The increase observed should be biologically relevant and statistically significant relative to concurrent and historical control frequencies for MN-PCE and/or MN-NCE induction.

The test would be considered inconclusive if the levels of MN-PCE within any one dose group were increased above the established historical control frequencies for MN-PCE induction, but not high enough to meet the criteria for a positive response. That is an increase up to 10% over the maximum negative control frequency for a group of animals.

5. Statistics - “Prior to statistical analysis the MN-PCE data were transformed by adding one to each individual value before taking the square root. These transformed values were analyzed using analysis of variance (ANOVA) techniques for males and females separately. Following the ANOVA, the SYN545192 treated groups were compared to the control using Dunnett’s tests at the 5% significance level”

II. Reported Results

A. Preliminary toxicity assay – In the second phase of the toxicity study, male rats received oral doses of SYN545192 at 175 mg/kg/day. Severe clinical signs were observed including: hunched posture, staggering, subdued behavior, piloerection, laboured breathing, and prostration. Due to the severity of the clinical observations, one female was humanely killed.
Based on these toxicity investigations, the maximum tolerated dose of SYN545192 was judged to be 175 mg/kg/day for male rats and 75 mg/kg/day for female rats.

Table 1. Toxicity Study – Clinical Signs and Deaths

Group No.	Dose Level (mg/kg/day)	Animal Numbers	Clinical Signs Observed (in one or more animals per group)	Deaths
1 (Males)	75	1M-3M	**Day 1:** NAD **Day 2:** NAD **Days 3-4:** NAD	0 0 0
1 (Females)	175	4F-6F	**Day 1:** Hunched (4F-6F), Staggering (4F - 6F), Subdued Behaviour (4F - 6F), Piloerection (4F, 6F), Laboured Breathing (4F, 6F) **Day 2:** Hunched (4F - 6F), Subdued Behaviour (4F - 6F) **Day 3:** NAD	0 0 0
2 (Males)		7M-9M	**Day 1:** Hunched (9M), Staggering (7M), Subdued Behaviour (7M, 9M) **Day 2:** Hunched (9M) **Days 3-4:** NAD	0 0 0
2	100	10F-12F	**Day 1:** Hunched (11F, 12F), Staggering (11F, 12F),	1

(Females)			Subdued Behaviour (11F, 12F), Piloerrection (12F) Laboured Breathing (12F), Prostration (12F), HK(12F) **Day 2:** Hunched (10F, 11F) **Days 3-4:** Hunched (11F)	0 0

NAD = No abnormalities detected
HK = Humanly Killed

B. <u>Micronucleus assay</u> – The frequency of MN-PCE in the high and low dose males (175 and 43.8 mg/kg/day respectively) was within the normal range. In the males treated with 87.5mg/kg/day and the females treated with 75 mg/kg/day, MN-PCE frequencies of 0.09% were observed. These responses were just over the borderline for a positive response when compared to the historical data (0.03-0.08%), but these increases are of no biological significance. There was no indication of bone marrow toxicity in any of the test item dose groups. The positive control group produced a significant increase.

Table 2. Summary of results – main assay (mean ± SD)

Treatment	Dose (µg/mL)	No. rats scored/ Sex	Erythrocytes				PCE/NCE
			Normochromatic cells (NCE)	Polychromatic cells (PCE)			
			No. of MN-NCE	PCE analysed	No. Of MN-PCE	% MN-PCE	
1% CMC with 0.1% TWEEN 80	10 mL/ kg/day	5 M	5	10015	7	0.07	0.62±0.11
		10 M	5	10008	6	0.06	0.65±0.14
		5 F	10	20023	13	0.06	0.64±0.12
SYN545192	43.8 mg/kg/day	5M	5	10012	1	0.01	0.64±0.19
SYN545192	87.5 mg/kg/day	5M	3	10009	9	0.09	0.69±0.18
SYN545192	175 mg/kg/day	5M	8	10016	6	0.06	0.56±0.11
SYN545192	75 mg/kg/day	5F	9	10007	9	0.09	0.55±0.15
Cyclophosphamide	50 mg/kg/day	5M	93$^{\alpha}$	10000	241	2.41$^{\beta}$	0.33±0.07

α: Evident response in NCE
β: Positive response in PCE
MN-PCE: Micronucleated PCE
NCE: Normochromatic erythrocytes
MN-NCE: Micronucleated NCE

III. Discussion

A. <u>Investigators' conclusions</u> – "It was concluded that SYN545192 did not induce micronuclei in bone marrow cells when tested to the maximum tolerated dose of 175 mg/kg/day in male and 75 mg/kg/day in female Wistar (Han) rats using a 0 h + 24 h oral dosing and 48 h sampling regimen"

B. <u>Reviewer comments</u> – The reviewer agrees with the study author's conclusion. SYN545192 did not induce micronuclei in bone marrow cells when tested to the maximum tolerated dose of 175 mg/kg/day in male and 75 mg/kg/day in female Wistar (Han) rats. There were no animal deaths noted during the study. The positive control induced the appropriate response.

C. <u>Study deficiencies</u> – None

B.6.5.1 Carcinogenicity study in the rat

Report:	IIA 5.5.2/01. Mackay C, 2012a. SYN545192: 104 week rat dietary carcinogenicity study with combined 52 week toxicity study. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Laboratory Report No. 30797, 02 February 2012. Unpublished. (Syngenta File No. SYN545192_10183). EPA MRID No. 48604446 (combined report)

Guidelines

Combined chronic toxicity/carcinogenicity, Rat (feeding)

OECD 453 (2009): OPPTS 870.4300 (1998): 96/54/EC B.33 (2004): JMAFF No.12-Nousan-8147 (2000)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

Dose Level Selection

Dose levels for this study were based upon an evaluation of results from a 90 day repeat oral study (SYN545192 – 90 Day Dietary Study in the Rat, Charles River Study No. 459292). Dose levels of 600 and 400 ppm were selected as the high dose for the males and females respectively, based on reductions in body weight and body weight gain, and histopathological changes seen in the liver at 750 and 1500 ppm. The magnitude of the decrease in body weight gain at 1500 ppm and 750 ppm in the 90 day study was considered to be in excess of that which could be tolerated over a 2 year period. The 90 day study also showed a clear sex difference for effects on body weight gain, with a more pronounced effect observed in females. On this basis, the top dose was selected at 600 ppm for males and 400 ppm in females. At these dose levels, a decrease in body weight gain of between 10 and 20% was expected. Dose levels of 25 and 100 ppm were selected, for the low and intermediate dose levels respectively. The dose of 100 ppm was selected to explore the dose-response of effects seen at the higher dose group. No adverse effects were expected at 25 ppm.

The selection of dose levels for this 104-week study in rats was made following consultation with a Dose Adequacy Review Team (DART) of the US Environmental Protection Agency. In the DART meeting on March 10, 2009, Syngenta presented the results of the 90-day rat study and its proposal to select dose levels of 0, 25, 100 and 600 ppm for males, and 0, 25, 100 and 400 ppm for females. The EPA DART subsequently acknowledged its agreement with the proposed dose levels for this study. [1]

[1] Kidwell, J. (2009). SYN545192 (HAMBRA): Report of the Dose Adequacy Review Team (DART) – Concurrence with Dose Selection for the 2-year Chronic Toxicity/Carcinogenicity Study in Rats. US Environmental Protection Agency. DP Barcode D363011. May 14, 2009.

EXECUTIVE SUMMARY

In a combined chronic toxicity and carcinogenicity study with SYN545192 (purity 97.0%), four groups of Han Wistar rats/sex were assigned to the carcinogenicity study and fed diets containing 0, 25, 100 or 600 (males), 400 (females) ppm SYN545192 for at least 104 consecutive weeks. In addition, a chronic toxicity study (comprising a further 4 groups of 12 rats/sex) was included and dosed in an identical fashion for a period of 52 consecutive weeks.

Clinical observations, body weight, food consumption, haematology, coagulation, clinical chemistry, organ weights, gross necropsy and histopathology were assessed in all groups. Urinalysis and ophthalmoscopy were assessed in the carcinogenicity groups only. A detailed functional observation battery assessment was made in the toxicity groups only. All surviving carcinogenicity and toxicity study animals were terminated after the completion of 104 or 52 weeks of treatment respectively and underwent a detailed necropsy examination with a comprehensive histopathological evaluation.

Body weight and body weight gains were decreased throughout treatment at 600/400 ppm in males and females, respectively. Food consumption was decreased throughout treatment at 400 ppm in females and sporadically in males at 600 ppm. Food utilization was decreased in both sexes at 600/400 ppm. In the clinical and detailed function observations, there was a slight increase in animals exhibiting no reaction to the tactile stimulus at 600/400 ppm and females at 400 ppm exhibited a slower tail flick response and were found to exhibit hunched/held low body posture, piloerection, staining on fur, thin appearance and rolling gait.

Changes to the liver occurred at 600 ppm in males and 400 ppm in females. Relative liver weights were increased in males following 104 weeks of treatment. In the toxicity phase, males and females exhibited centrilobular hypertrophy and females exhibited centrilobular hepatocyte pigmentation. In the carcinogenicity phase, males and females exhibited centrilobular hypertrophy, males exhibited pale foci grossly and eosinophilic cell foci and hepatocyte vacuolation histopathologically, and females exhibited an increase in hepatocyte centrilobular pigment.

Other microscopic changes were seen in 400 ppm females following 104 weeks of treatment and consisted of increased tubular cell pigment deposits in the kidneys, focal c-cell hyperplasia of the thyroid, lobular hyperplasia of the mammary glands and pigmented macrophages in the spleen.

Neoplastic changes consisted of an increase in thyroid follicular cell adenomas in 600 ppm males.

The LOAEL is 600/400 ppm (30.17/27.44 mg/kg bw per day) in males and females respectively, based on decreased body weights, body weight gains, food consumption, food utilization, lack of response to tactile stimulus and liver changes in males and females, thyroid follicular cell adenomas in males and hunched or held low body position, piloerection, staining on fur and thin appearance, slow reaction to tail flick and rolling gait, increased tubular cell pigment deposits in the kidneys, focal c-cell hyperplasia of the thyroid, lobular hyperplasia of the mammary glands and pigmented macrophages in the spleen in females. The NOAEL is 100 ppm (4.88/6.66 mg/kg bw per day).

Classification: Acceptable-guideline

MATERIALS AND METHODS

Materials:

Test Material:	SYN545192
Description:	Technical, beige powder
Lot/Batch number:	SMU9BP005
Purity:	97.0% a.i
CAS#:	Not reported
Stability of test compound:	Reanalysis date end February 2013 (stored <30°C)

Vehicle and/or positive control: The test substance was administered in Rat and Mouse (Modified) No.1 (RM1) Diet SQC Expanded (Fine Ground).

Test Animals:	
Species	Rat
Strain	Han Wistar rats (CrL:WI(Han))
Age/weight at dosing	Approximately 6 weeks / 129-196 g (males), 101-159 g (females)
Source	Charles River UK Limited, Margate, Kent, UK
Housing	Up to 4/same sex/ cage in suspended polycarbonate cages (overall dimensions 61 x 43.5 x 24 cm) with stainless steel grid tops, solid bottoms and separate stainless steel food hoppers
Acclimatisation period	15 days
Diet	Rat and Mouse (modified) No. 1 Diet SQC Expanded (supplied by Special Diets Services Limited, 1 Stepfield, Witham, Essex, UK) *ad libitum*
Water	Domestic water *ad libitum*
Environmental conditions	Temperature: 18-25°C Humidity: 26-77% Air changes: Minimum of 15/hour Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

In-life dates: Start: 18 March 2009 End: 17 August 2011

Animal assignment: On arrival from the suppliers, the animals (treated and controls) were allocated to cages on racks. Cages were racked by treatment group and vertically throughout the rack. Each month, from Week 3, each column of cages on a rack was moved one position along the rack. During pre-trial, body weights were checked to ensure that all groups had a similar group mean body weight for each sex and all were found to be within the 20% limit of variation. Animals were allocated to dose groups as in the table below:

Table B.6.5.1/01: Study design

Test group	Dietary concentration (ppm)	Animal numbers			
		Carcinogenicity study		Toxicity study	
		Males	Females	Males	Females
Control	0	52	52	12	12
Low	25	52	52	12	12
Mid	100	52	52	12	12
High (males)	600	52	-	12	-
High (females)	400	-	52	-	12

Diet preparation and analysis: A 200 g stock was prepared by mixing the test substance with the required amount of untreated control diet in an automated mortar and pestle until visibly homogeneous. A high level premix at 5000 ppm was prepared by adding the appropriate amount of blank RM1 diet to the 200 g stock and mixing. Diet formulations for high and intermediate dose groups (600, 400 and 100 ppm) were prepared as dilutions from the high level premix. A low level premix at 600 ppm was prepared as a dilution from the high level premix. Diet formulations for the low dose group (25 ppm) were prepared as a dilution from the low level premix. Blank diet (without the test substance) was prepared for control animals. Diet formulations were prepared at appropriate intervals (typically every 2 weeks).

The diet formulations were analysed for achieved concentration and homogeneity at intervals throughout the study.

Analysis results: For diets prepared for use in weeks 1/2, 13/14, 27/28, 39/40, 51/52, 65/66, 79/80, 91/92 and 103/104, the mean test substance concentration at each level was between -10.8% and 5.8% of the nominal concentration, with a maximum variability between replicate samples of 8.6%, indicating that the diets were correctly prepared. SYN545192 was not detected in control diet at any time-point.

Stability results: Stability data for diets at 10-5000 ppm was confirmed for 30 days when stored at ambient temperature in the dark, following work carried out in the Analytical Chemistry Services of Charles River, Edinburgh under a separate protocol.

Observations: Animals were examined/observed twice daily for viability and once weekly were given a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.

Body weight: The bodyweight of each rat was recorded once each week, pre-trial up to week 14, and once every 2 weeks from week 16 until the end of treatment.

Food consumption, utilisation and test substance intake: The quantity of food consumed by each cage of animals was measured and recorded once each week pre-trial until week 14 and once every 2 weeks from week 16 until the end of treatment.

Food utilisation was calculated for weeks 1-4, 5-8, 9-13 and 1-13 by dividing (cage mean weight gain x 100) by cage total food consumption.

The amount of test substance ingested was calculated at regular intervals during treatment using the following formula:

$$\text{Achieved intake (mg/kg/day)} = \frac{\text{Nominal Concentration (ppm)} \times \text{Food Consumption (g/day)}}{\text{Mid-point Body Weight (g)}}$$

Water consumption: Qualitatively monitored by visual inspection of the water bottles on a weekly basis throughout the study.

Ophthalmoscopic examination: The eyes were examined using an indirect ophthalmoscope following application of a mydriatic agent. The anterior, lenticular and fundic regions were examined from all carcinogenicity animals during pre-trial and all surviving carcinogenicity control and high dose animals during weeks 50 and 102.

Functional observation battery: Once during week 51/52, a more detailed examination was made of all toxicity study animals as detailed below. The observations were made by one observer who was 'blind' with respect to the animals' treatment and were performed at an approximately standardised time of day.

Cageside observations:

Posture/condition on first approach (animal undisturbed), checking for: prostration, lethargy, writhing, circling, breathing abnormalities, gait abnormalities, tremor, fasciculation, convulsions, biting (of cage components or self mutilating), vocalisations, piloerection.

Ease of removal from the cage.

Body temperature.

Condition of the eyes, checked for: pupillary function, miosis, mydriasis, exophthalmos, encrustation, lachrymation.

Condition of the coat.

Presence of salivation.

Overall ease of handling.

Observations in a standardised area (2 minute observation):

Latency (time to first locomotory movement), level of mobility, rearing, grooming, urination/defecation, arousal (level of alertness), posture, tremor/convulsions, vocalisation, piloerection – recorded as for cageside observations, palpebral closure, gait abnormalities, stereotypy (excessive repetition of behaviours) and/or unusual behaviours.

Functional tests

Reaction to sudden sound (click above the head), reaction to touch on the rump with a blunt probe, grip strength (fore and hind limbs), pain perception, landing foot splay, motor activity.

Other physical/functional abnormalities

Any other abnormality not already recorded in the above screening battery.

Clinical pathology: Blood was collected via the tail vein without anaesthesia from 13 rats/sex/group from the carcinogenicity study at weeks 14, 27, 53 and 79 and all toxicity study animals during week 52. Blood samples were obtained, via orbital sinus under isoflurane anaesthesia, from all surviving carcinogenicity study animals before necropsy.

Haematology: The following parameters were examined:

haemoglobin
haematocrit
red blood cell count
mean cell volume
mean cell haemoglobin
activated partial thromboplastin time
mean cell haemoglobin concentration
platelet count
total white cell count
differential white cell count
prothrombin time

Clinical chemistry: The following parameters were examined:

urea
creatinine
glucose
albumin
globulin
total protein
cholesterol
triglycerides
total bilirubin
creatine phosphokinase activity
alkaline phosphatase activity (ALP)
aspartate aminotransferase activity (AST)
alanine aminotransferase activity (ALT)
gamma-glutamyl transferase activity
albumin/globulin ratio
calcium
phosphorus (as phosphate)
sodium
potassium
chloride

Urinalysis: Urine was collected over a 4 hour period from 13/rats/sex/group from the carcinogenicity study animals during weeks 13, 26, 52, 78 and 104. The animals were housed individually in metabolism cages and were deprived of food and water. The following parameters were evaluated:

volume
colour
specific gravity
pH
urobilinogen
microscopy of spun sediment
glucose
ketones
protein
bilirubin
blood pigments

Toxicokinetics: All toxicity study animals were bled (0.5 mL at each time point) for toxicokinetic sampling at 07:00, 11:00 and 19:00 hours during week 49 via the tail vein. For both the toxicity study and carcinogenicity study animals, approximately 2 mL of blood was

collected from all surviving animals, at the same time as the clinical pathology samples, via the orbital sinus, under isoflurane anaesthesia, before necropsy. All blood samples were processed to plasma via centrifugation and stored, deep frozen, for possible future analysis.

Investigations *post-mortem:* After at least 52 or 104 weeks (toxicity and carcinogenicity study animals, respectively) of treatment all surviving animals were killed in random order by exposure to a rising concentration of carbon dioxide and had their terminal body weight recorded, followed by exsanguination.

Macroscopic examination: All animals were examined *post mortem*. This involved an external observation and an internal examination of all organs and structures.

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands
brain
epididymides
heart
kidneys
liver
ovaries
spleen
testes
uterus

Paired organs were weighed separately and the sum used for reporting purposes.

Tissue submission: The following tissues from all toxicity and carcinogenicity animals, surviving to scheduled termination, were examined *in situ*, removed and processed to paraffin wax blocks, stained with haematoxylin and eosin and examined histopathologically:

gross lesions including masses (with lymph nodes local to masses)
adrenal gland
aortic arch
brain (forebrain, midbrain, cerebellum, pons)
epididymis
bone marrow (femur)
eyes
femur (including knee joint and bone marrow)
stomach
duodenum
jejunum
ileum
caecum
colon
rectum
Harderian gland
heart
kidney
lachrymal gland
optic nerves
ovary
oviduct
pancreas
pharynx
pituitary gland
prostate gland
nerve - sciatic
seminal vesicle
skin and mammary gland
spinal cord (cervical, midthoracic, lumbar)
spleen
sternum (including bone marrow)
lymph node - submandibular
salivary gland
testis
thigh muscle
thymus

larynx	thyroid gland with parathyroid
liver	tongue
lung	trachea
marrow smear (femur)	urinary bladder
lymph node - mesenteric	uterus
oesophagus	vagina

In addition, from all toxicity and carcinogenicity animals surviving to scheduled termination, multiple samples of liver (8 x *ca* 150 mg) were taken from two 5 mm sections of the lateral lobe. The samples were snap frozen in liquid nitrogen in individual RNA-ase free tubes. All samples were taken as quickly as possible and stored at *ca* -70°C pending possible future analysis. Duplicate femoral bone marrow smears were taken at necropsy. One bone marrow smear was stained using May-Grunwald-Giemsa as the Romanowsky stain and for toxicity study animals, the smears were evaluated qualitatively. For carcinogenicity study animals, the smears were not evaluated. The second, unstained, smear was retained in the pathology department as a contingency.

Statistics: Body weight, cumulative body weight gain, food consumption, food utilisation, haematology, coagulation, clinical chemistry, quantitative urinalysis values, quantitative functional observation battery measurements, motor activity and organ weight data were analysed using a parametric ANOVA and pairwise comparisons made using the Dunnett's t-test. The following pairwise comparisons were performed: Control Group vs Low Dose, Control Group vs Intermediate Dose, Control Group vs High Dose. Organ weights were also analysed by analysis of covariance (ANCOVA) using terminal kill body weight as covariate followed by a pairwise comparison using Dunnett's t-test. Kaplan-Meier survival estimates were calculated separately for each sex and treatment group. Histological incidence data were analysed using Fisher's Exact Probability Test. Pairwise comparisons of the incidence of tumour and histological lesions was made using Fisher's Exact Probability test function within PLACES 2000. Further analysis was performed using Peto's time adjusted methods. Methods used for the age-adjusted analysis of fatal and non-fatal tumours were based on the IARC guidelines.

All statistical tests were two sided and performed at the 5% and 1% levels.

RESULTS AND DISCUSSION

Mortality:

Toxicity study: There were no unscheduled deaths in the toxicity phase.

Carcinogenicity study: There was no treatment-related effect on mortality.

Clinical observations:

Toxicity study: There was a slight increase in bald areas at 100 and 400 ppm females in the toxicity phase of the study, but the observation was not seen in the carcinogenicity phase and was not considered attributable to treatment.

Carcinogenicity study: In the carcinogenicity phase, there was a slight increase in clinical observations related to decreased body weights and reduced body condition at the high-dose in females. This consists of hunched/held low body posture, piloerection, staining on fur and thin appearance. Males at 600 ppm also exhibited slightly higher incidences of agitation. There was a very slight increase in rolling gait in females at 400 ppm that was considered to be treatment-related due to the same clinical sign being noted in the mouse oncogenicity study.

Table B.6.5.1/02: Intergroup comparison of selected clinical observations – toxicity study (52 weeks)[a]

	Dietary Concentration of SYN545192 (ppm)							
	Males				Females			
	0	**25**	**100**	**600**	**0**	**25**	**100**	**400**
(Number of animals)	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)
Area(s) of sparse hair	5	2	10	9	3	8	9	7
Bald area(s)	1	0	0	1	0	0	4	5

[a] Data obtained from pages 87 – 88 of the study report

Table B.6.5.1/03: Intergroup comparison of selected clinical observations – carcinogenicity study (104 weeks)[a]

	Dietary Concentration of SYN545192 (ppm)							
	Males				Females			
	0	**25**	**100**	**600**	**0**	**25**	**100**	**400**
(Number of animals)	(52)	(52)	(52)	(52)	(52)	(52)	(52)	(52)
Body (hunched/held low)	6	6	6	6	17	10	14	**20**
Piloerection	4	5	1	5	9	6	11	**13**
Staining on fur (dorsal abdomen/dorsal neck/dorsal surface/dorsal thorax/ears/eye(s)/feet/head/limb(s)/ muzzle/whole body/ventral abdomen/ extremities)	33	30	31	34	19	15	22	**28**
Thin	1	2	3	3	1	1	2	**6**
Agitated	1	1	3	5	0	2	1	2
Areas of sparse hair	28	31	26	25	34	42	32	38
Bald area(s)	4	8	9	9	8	6	4	6
Rolling gait	4	7	3	7	15	12	15	**21**
Walking on tip toes	0	0	0	0	5	4	3	7

[a] Data obtained from pages 89 – 96 of the study report

Bolded values determined to be treatment-related

Bodyweight and weight gain: High-dose males and females exhibited decreased body weight and body weight gains compared to controls throughout treatment.

Table B.6.5.1/04: Intergroup comparison of body weight and bodyweight gain – carcinogenicity and toxicity studies combined (g) - selected weeks[a]

	Dietary Concentration of SYN545192 (ppm)							
	Males				Females			
Weeks	**0**	**25**	**100**	**600**	**0**	**25**	**100**	**400**
	Body weights							
-1	123 ± 12	122 ± 11	122 ± 11	123 ± 10	110 ± 8	110 ± 10	110 ± 7	109 ± 11
0	166 ± 15	164 ± 14	164 ± 14	163 ± 13	132 ± 9	133 ± 11	132 ± 8	131 ± 12
1	207 ± 18	205 ± 16	205 ± 17	**196 ± 15**** (↓5)	155 ± 10	156 ± 12	155 ± 9	150 ± 12* (↓3)
2	238 ± 20	237 ± 19	233 ± 20	**223 ± 17**** (↓6)	168 ± 10	168 ± 13	166 ± 11	162 ± 13** (↓4)
10	360 ± 38	361 ± 39	358 ± 38	**334 ± 31**** (↓7)	222 ± 14	223 ± 18	218 ± 16	**199 ± 15**** (↓10)
32	462 ± 53	465 ± 54	459 ± 50	**426 ± 39**** (↓8)	260 ± 20	256 ± 20	255 ± 22	**228 ± 18**** (↓12)
52	524 ± 62	529 ± 63	521 ± 61	**481 ± 44**** (↓8)	287 ± 32	288 ± 30	282 ± 34	**238 ± 21**** (↓17)
84	606 ± 67	601 ± 82	593 ± 75	**542 ± 57**** (↓11)	340 ± 49	341 ± 52	338 ± 57	**264 ± 34**** (↓22)
104	615 ± 75	608 ± 79	613 ± 78	**548 ± 62**** (↓11)	366 ± 56	356 ± 54	353 ± 65	**275 ± 38**** (↓25)
	Bodyweight gains							
0-1	41 ± 5	41 ± 5	40 ± 5	**33 ± 5**** (↓20)	22 ± 4	23 ± 4	23 ± 4	**19 ± 4**** (↓14)
0-3	99 ± 13	100 ± 12	95 ± 13	**82 ± 13**** (↓17)	48 ± 5	48 ± 7	45 ± 8	**39 ± 7**** (↓19)
0-10	195 ± 28	198 ± 31	194 ± 30	**171 ± 26**** (↓12)	90 ± 8	90 ± 13	85 ± 12	**68 ± 11*** (↓24)
0-32	296 ± 45	301 ± 46	295 ± 43	**263 ± 35**** (↓11)	127 ± 16	123 ± 15	123 ± 17	**97 ± 13**** (↓24)
0-52	358 ± 55	366 ± 56	357 ± 54	**318 ± 41**** (↓11)	155 ± 28	155 ± 25	150 ± 29	**107 ± 16**** (↓31)
0-84	439 ± 61	437 ± 74	430 ± 70	**380 ± 53**** (↓13)	207 ± 47	208 ± 48	205 ± 52	**133 ± 29**** (↓36)
0-104	448 ± 70	444 ± 71	450 ± 73	**386 ± 59**** (↓14)	233 ± 55	223 ± 52	221 ± 59	**144 ± 33**** (↓38)

[a] Data obtained from pages 97 – 116 of the study report

* Statistically significant difference from control group mean, $p<0.05$ (Dunnett's test, 2-sided)

** Statistically significant difference from control group mean, $p<0.01$ (Dunnett's test, 2-sided)

Food consumption and compound intake: Food consumption was decreased compared to controls in males in a statistically significant manner throughout treatment at 600 ppm, but only decreased to a biologically significant amount at weeks 7 and 34. In females, food consumption was decreased to a statistically and biologically significant degree at weeks 5-9, 11-12, 16, 24, 28, 34-40, 44-46, 54-56, 64-66, 76, 80-96 and 100.

There were statistically significant changes in food consumption at 25 and 100 ppm, however, the changes were sporadic and determined to not be related to treatment.

Table B.6.5.1/05: Intergroup comparison of food consumption (g/animal/day) – carcinogenicity and toxicity studies combined (g) - selected weeks[a]

	Dietary Concentration of SYN545192 (ppm)							
	Males				Females			
Week	**0**	**25**	**100**	**600**	**0**	**25**	**100**	**400**
1	21.2 ± 1.3	22.3 ± 1.3* (↑5)	21.9 ± 1.2	20.6 ± 1.0	15.5 ± 1.0	15.4 ± 1.0	15.7 ± 0.9	14.6 ± 1.1* (↓6)
2	22.3 ± 1.4	22.8 ± 0.9	22.0 ± 1.1	20.9 ± 1.2** (↓6)	16.2 ± 1.8	16.5 ± 1.3	16.1 ± 1.4	15.7 ± 1.1
4	21.7 ± 1.3	22.8 ± 1.2 (↑6)	22.8 ± 1.5 (↑7)	21.7 ± 1.5	16.9 ± 1.1	17.2 ± 0.9	17.3 ± 0.8	15.4 ± 1.0** (↓9)
5	21.4 ± 1.6	22.4 ± 1.0	21.7 ± 1.4	20.6 ± 1.4	17.3 ± 1.0	17.5 ± 1.0	17.2 ± 0.9	**15.4 ± 1.0** (↓11)**
6	23.1 ± 1.7	23.5 ± 1.1	22.9 ± 1.3	21.3 ± 1.6** (↓8)	18.3 ± 1.0	18.6 ± 1.5	18.1 ± 1.3	**16.1 ± 1.8** (↓12)**
7	23.6 ± 1.7	23.7 ± 1.7	23.3 ± 1.2	**21.2 ± 1.3** (↓10)**	18.7 ± 1.7	18.5 ± 1.6	18.1 ± 1.9	**15.8 ± 1.8** (↓16)**
8	21.2 ± 1.2	21.5 ± 1.5	21.1 ± 1.1	20.1 ± 1.2* (↓5)	20.8 ± 2.1	21.6 ± 2.0	21.2 ± 2.0	**17.8 ± 1.5** (↓14)**
9	21.6 ± 1.0	22.3 ± 1.2	21.9 ± 1.1	20.3 ± 0.8** (↓6)	20.5 ± 2.7	21.1 ± 2.4	21.9 ± 2.5	**16.9 ± 2.5** (↓18)**
10	23.5 ± 1.4	23.8 ± 1.6	23.3 ± 1.9	21.3 ± 1.4** (↓9)	16.9 ± 1.3	18.4 ± 2.4	18.8 ± 2.1* (↑11)	15.8 ± 2.5
11	23.2 ± 1.4	22.7 ± 3.5	23.1 1.3	22.5 ± 1.7	19.4 ± 2.1	18.8 ± 2.7	18.1 ± 1.5	**15.2 ± 1.7** (↓22)**
34	21.9 ± 1.3	21.7 ± 1.2	21.4 ± 1.2	**19.8 ± 1.4** (↓10)**	17.7 ± 1.5	17.2 ± 1.3	16.6 ± 1.0* (↓6)	**14.8 ± 0.6** (↓16)**
42	20.4 ± 1.2	20.8 ±1 .0	19.7 ± 1.2	19.1 ± 1.0** (↓6)	15.8 ± 1.2	16.6 ± 1.3	15.3 ± 1.3	14.9 ± 1.4
44	20.8 ± 1.4	21.4 ± 1.2	19.8 ± 1.2	20.2 ± 1.4	16.7 ± 1.3	16.2 ± 1.1	15.3 ± 1.0** (↓8)	**14.6 ± 0.8** (↓13)**
46	19.6 ± 1.2	20.0 ± 1.0	19.3 ± 1.3	19.0 ± 0.8	17.3 ± 2.1	17.3 ± 1.4	16.3 ± 2.3	**14.4 ± 2.1** (↓17)**
90	21.0 ± 1.2	20.7 ± 2.2	22.5 ± 1.5	21.1 ± 1.9	16.8 ± 1.2	17.1 ±1 .4	16.4 ± 1.1	**14.9 ± 1.2** (↓11)**
100	21.4 ± 1.3	20.3 ±1 .2	21.0 ± 1.6	19.5 ± 1.5** (↓9)	16.7 ± 1.7	15.6 ± 1.4	16.0 ± 1.7	**13.8 ± 1.3** (↓17)**
104	20.8 ± 2.6	20.6 ± 2.7	22.8 ± 1.7	20.4 ± 2.8	16.3 ± 1.3	16.3 ± 1.4	18.6 ± 2.3* (↑14)	18.6 ± 3.6* (↑14)

[a] Data obtained from pages 117 – 126 of the study report

Bolded values determined to be treatment-related and adverse

* Statistically significant difference from control group mean, p<0.05 (Dunnett's test, 2-sided)

** Statistically significant difference from control group mean, p<0.01 (Dunnett's test, 2-sided)

Achieved dose levels for the full 104 week carcinogenicity study were calculated and mean values are shown below:

Table B.6.5.1/06: Mean Dose Received (mg/kg/day) - carcinogenicity study

SYN545192 (ppm)	25	100	600/400
Males	1.21	4.88	30.17
Females	1.65	6.66	27.44

Food utilisation: While food utilization was decreased to a statistically significant degree at 600 ppm in males in weeks 1 – 4 and 1 – 13, it was only reduced to a biologically significant degree in weeks 1 – 4. In 400 ppm females, food utilization was decreased to a biological and statistically significant degree in weeks 1 – 4, 9 – 13, and 1 – 13. Food utilization in both males and females at 100 ppm were reduced to a statistically significant degree in weeks 1 – 4; however, they were not reduced to a biologically significant degree.

Table B.6.5.1/07: Intergroup comparison of food utilisation (weight gained (g)/100g food) – carcinogenicity and toxicity studies combined (g) - selected weeks

	Dietary Concentration of SYN545192 (ppm)							
	Males				Females			
Weeks	**0**	**25**	**100**	**600**	**0**	**25**	**100**	**400**
1-4	19.7 ± 0.9	19.2 ± 0.8	18.8 ± 0.8** (↓5)	**16.7 ± 1.3** (↓15)**	13.0 ± 0.9	12.6 ± 0.9	12.1 ± 0.9* (↓7)	**10.7 ± 0.9** (↓18)**
5-8	9.2 ± 1.0	9.2 ± 1.3	9.3 ± 0.7	9.1 ± 0.9	4.5 ± 0.4	4.7 ± 0.8	4.3 ± 0.6	4.2 ± 0.5
9-13	5.6 ± 0.4	5.3 ± 0.5	5.4 ± 0.4	5.8 ± 0.5	2.7 ± 0.6	2.6 ± 0.5	2.6 ± 0.7	**2.2 ± 0.4* (↓19)**
1-13	11.0 ± 0.5	10.8 ± 0.7	10.8 ± 0.5	10.3 ± 0.6** (↓6)	6.2 ± 0.5	6.1 ± 0.6	5.8 ± 0.4	**5.3 ± 0.3** (↓15)**

[a] Data obtained from pages 127 – 128 of the study report

Bolded values determined to be treatment-related

* Statistically significant difference from control group mean, $p<0.05$ (Dunnett's test, 2-sided)

** Statistically significant difference from control group mean, $p<0.01$ (Dunnett's test, 2-sided)

Water consumption: Water consumption was monitored visually and not quantitatively. According to the study authors, there was no observable difference in water consumption between the groups.

Ophthalmoscopic examination: There were no treatment-related effects on ophthalmoscopic parameters.

Functional observation battery:

Motor activity: There was no effect on motor activity or habituation.

Detailed functional observations: There was a slight increase in the number of animals exhibiting no reaction to the tactile stimulus in males and females at 600/400 ppm. In females, there was also a 31% increase in the time to tail flick reaction at 400 ppm.

Table B.6.5.1/08: Intergroup comparison of select detailed functional observations

	Dietary Concentration of SYN545192 (ppm)							
	Males				Females			
Observation	**0**	**25**	**100**	**600**	**0**	**25**	**100**	**400**
Tactile stimulus, normal reaction	12	12	11	10	12	12	12	10
Tactile stimulus, no reaction	0	0	1	**2**	0	0	0	**2**
Tail Flick (s)	4.1 ± 1.1	4.2 ± 1.3	3.7 ± 0.8 (↓10)	3.5 ± 0.6 (↓15)	3.2 ± 0.7	3.5 ± 0.7 (↑9)	3.7 ± 0.5 (↑16)	**4.2 ± 0.9** (↑31)**

[a] Data obtained from pages 129 - 132 of the study report

Bolded values determined to be treatment-related

* Statistically significant difference from control group mean, p<0.05 (Dunnett's test, 2-sided)

** Statistically significant difference from control group mean, p<0.01 (Dunnett's test, 2-sided)

Haematology: There was a sporadic but statistically significant decrease in haemoglobin, red blood cell counts and haematocrit in females at 400 ppm; however, the magnitude of the change never reached biological significance and was not considered adverse.

Blood clinical chemistry: In the clinical chemistry parameters, alkaline phosphatase activity (ALP), aspartate aminotransferase activity (AST), and alanine aminotransferase activity (ALT) were all decreased to some extent in the high-dose animals. However, the magnitude of decrease does not consistently reach levels that would be considered adverse for this type of clinical chemistry parameter and there is inconsistent dose response in the latter half of the study when there were changes to the liver noted. The adversity of these changes are unknown.

Table B.6.5.1/09: Intergroup comparison of selected clinical chemistry parameters throughout the study

Clinical Chemistry Parameter	Week	Dietary Concentration of SYN545192 (ppm)							
		Males				Females			
		0	**25**	**100**	**600**	**0**	**25**	**100**	**400**
ALP	13	93 ± 25	89 ± 12	88 ± 20	69 ± 17** (↓26)	43 ± 10	46 ± 11	45 ± 9	35 ± 8 (↓19)
	27	71 ± 17	70 ± 12	71 ± 16	54 ± 8* (↓24)	39 ± 7	38 ± 7	37 ± 7	29 ± 11* (↓26)
	52	76 ± 18	59 ± 10*	67 ± 15	47 ± 9** (↓38)	40 ± 11	34 ± 9	36 ± 10	28 ± 7** (↓30)
	53	69 ± 19	72 ± 12	75 ± 21	48 ± 8* (↓30)	36 ± 8	37 ± 10	36 ± 10	28 ± 12 (↓22)
	79	73 ± 27	73 ± 13	70 ± 22	46 ± 10** (↓37)	36 ± 12	36 ± 9	34 ± 10	22 ± 4** (↓39)
	Terminal	73 ± 21	86 ± 91	73 ± 22	49 ± 14 (↓33)	45 ± 14	43 ± 15	37 ± 14* (↓18)	30 ± 12** (↓33)
ALT	13	45 ± 8	42 ± 7	40 ± 6	40 ± 7 (↓11)	41 ± 7	42 ± 8	39 ± 6	38 ± 9 (↓7)

	27	51 ± 11	41 ± 8** (↓20)	40 ± 7** (↓22)	37 ± 6** (↓27)	59 ± 13	57 ± 28 (↓3)	46 ± 13 (↓22)	45 ± 10 (↓24)
	52	54 ± 17	48 ± 25	50 ± 17	44 ± 13 (↓19)	62 ± 23	56 ± 20	62 ± 28	49 ± 15 (↓21)
	53	49 ± 16	52 ± 16	49 ± 11	35 ± 5* (↓29)	68 ± 28	87 ± 110 (↑28)	52 ± 11 (↓24)	47 ± 16 (↓31)
	79	67 ± 37	63 ± 31	57 ± 14	50 ± 22 (↓25)	81 ± 50	87 ± 58	61 ± 17 (↓25)	52 ± 10 (↓36)
	Terminal	65 ± 39	57 ± 24	57 ± 20	53 ± 24 (↓18)	73 ± 28	64 ± 27	64 ± 19	69 ± 29 (↓5)
AST	13	74 ± 14	74 ± 19	68 ± 11	60 ± 7* (↓19)	68 ± 8	67 ± 6	64 ± 8	58 ± 7** (↓15)
	27	64 ± 8	63 ± 11	58 ± 5	55 ± 4* (↓14)	69 ± 4	85 ± 59 (↑23)	58 ± 9 (↓16)	61 ± 10 (↓12)
	52	72 ± 19	69 ± 30	68 ± 17	72 ± 14 (0)	94 ± 33	93 ± 46	94 ± 33	77 ± 16 (↓18)
	53	70 ± 21	69 ± 15	66 ± 10	55 ± 4* (↓21)	108 ± 64	143 ± 212 (↑32)	74 ± 24 (↓31)	68 ± 16 (↓37)
	79	89 ± 41	78 ± 19 (↓12)	68 ± 13 (↓24)	60 ± 11* (↓33)	152 ± 131	163 ± 199	91 ± 39 (↓40)	77 ± 24 (↓49)
	Terminal	88 ± 45	85 ± 56	73 ± 20	75 ± 24 (↓15)	124 ± 64	100 ± 37 (↓19)	103 ± 52 (↓17)	107 ± 45 (↓14)

[a] Data obtained from pages 161 – 184 of the study report

* Statistically significant difference from control group mean, $p<0.05$ (Dunnett's test, 2-sided)

** Statistically significant difference from control group mean, $p<0.01$ (Dunnett's test, 2-sided)

Urinalysis: There were no treatment-related changes to the urinalysis parameters.

Sacrifice and pathology:

Organ weights:

Toxicity study: There were no treatment-related changes to organ weight parameters following 52 weeks of treatment.

Carcinogenicity study: In high-dose males, absolute liver weights were unaffected by treatment, but relative liver weights were increased compared to controls. This is correlated with increased centrilobular hypertrophy in carcinogenicity-phase animals. While absolute liver weights are decreased in females, this was considered secondary to the decreased body weights.

Table B.6.5.1/10: Intergroup comparison of organ weight (g) - carcinogenicity study (104 weeks)

	Dietary Concentration of SYN545192 (ppm)							
	Males				Females			
	0	**25**	**100**	**600**	**0**	**25**	**100**	**400**
Body weight (g)	609 ± 75	596 ± 79	607 ± 79	**543 ± 62** (↓11)**	360 ± 57	349 ± 56	350 ± 67	**268 ± 37** (↓26)**
Absolute liver	17.38 ± 2.687	17.42 ± 2.56	18.25 ± 2.56	17.07 ± 2.42	10.78 ± 1.86 (↓2)	10.54 ± 2.04	10.98 ± 2.12	8.69 ± 1.34** (↓19)
Relative liver	2.857 ± 0.298	2.934 ± 0.326	3.023 ± 0.362	**3.147 ± 0.304 (↑10.1)**	3.012 ± 0.386 (↑10)	3.016 ± 0.307	3.149 ± 0.360	3.244 ± 0.305 (↑8)

* Statistically significant difference from control group mean, p<0.05 (Dunnett's test, 2-sided)

** Statistically significant difference from control group mean, p<0.01 (Dunnett's test, 2-sided)

Macroscopic findings: There were no treatment-related changes noted in the gross necropsy of the toxicity-phase animals.

In the carcincogenicity-phase animals, there was an increase in pale foci in the liver of high-dose males. There were also increases in skin discolouration and thyroid gland enlargement, in high-dose males. Thyroid enlargement occurred in the presence and absence of thyroid adenomas. There were no other histopathological correlations and the changes were not considered adverse.

Table B.6.5.1/11: Intergroup comparison of selected macroscopic findings – carcinogenicity study[a]

	Dietary Concentration of SYN545192 (ppm)							
	Males				Females			
	0	**25**	**100**	**600**	**0**	**25**	**100**	**400**
Liver (Number of animals)	(52)	(52)	(52)	(52)	(52)	(52)	(52)	(52)
Liver - Foci, pale	0	1	1	**6**	2	2	1	0
Skin (Number of animals)	(52)	(52)	(52)	(52)	(52)	(52)	(52)	(52)
Skin - Discolouration	8	5	9	17	5	5	7	4
Thyroid Gland (Number of animals)	(52)	(52)	(52)	(52)	(52)	(52)	(52)	(52)
Thyroid Gland - Enlargement, one/both	3	4	4	7	0	1	0	0

[a] Data obtained from pages 213 – 238 of the study report

Bolded values determined to be treatment-related

Microscopic findings:

Toxicity study: In the toxicity phase of the study, centrilobular hypertrophy was noted in males at 600 ppm and in females at 400 ppm. In addition, high-dose females exhibited centrilobular hepatocyte pigmentation.

Table B.6.5.1/12: Intergroup comparison of selected liver microscopic findings – toxicity study (52 weeks)

	Dietary Concentration of SYN545192 (ppm)							
	Males				Females			
	0	**25**	**100**	**600**	**0**	**25**	**100**	**400**
Liver (Number of animals)	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)
Centrilobular hypertrophy (total)	0	0	2	9**	0	0	1	10**
- minimal	0	0	2	3	0	0	0	3
- mild	0	0	0	4	0	0	1	6*
- moderate	0	0	0	2	0	0	0	1
Hepatocyte pigment, centrilobular (total)	0	0	0	0	0	0	1	6*
- minimal	0	0	0	0	0	0	1	6*

* Statistically significant difference from control group mean, p<0.05 (Fisher's Exact Test)

** Statistically significant difference from control group mean, p<0.01 (Fisher's Exact Test)

Carcinogenicity study:

Non-neoplastic findings: In the carcinogenicity phase of the study, the liver was still a target organ. Eosinophilic cell foci were noted in males at 600 ppm and there were very slight increases in centrilobular hepatocyte pigmentation in females at 400 ppm and hepatocyte vacuolation in males at 600 ppm. Centrilobular hypertrophy was noted at doses ≥ 100 ppm in males and at 400 ppm in females; however the change was considered adverse in high-dose animals only due to the lack of correlated organ, clinical chemistry and/or pathological changes.

In the kidneys, there was an increase in pigment deposits in the tubular cells at 400 ppm in females. The increase in focal c-cell hyperplasia in females at 400 ppm was considered treatment-related due to the increase in adenomas in males. There was an increase in lobular hyperplasia in the mammary glands of high-dose females and pigmented macrophages in the spleen at the same dose. There was an increase in focal ulcers in the non-glandular stomach at doses ≥ 100 ppm that may be related to the increased hyperplasia in the colon and caecum seen in the mouse oncogenicity study.

Table B.6.5.1/13: Intergroup comparison of selected microscopic findings – carcinogenicity study

	Dietary Concentration of SYN545192 (ppm)							
	Males				Females			
	0	**25**	**100**	**600**	**0**	**25**	**100**	**400**
Liver (Number of animals)	(52)	(52)	(52)	(52)	(52)	(52)	(52)	(52)
Liver - Eosinophilic cell focus (total)	1	5	4	**15****	2	0	3	5
- minimal	0	2	0	**2**	0	0	2	1
- mild	1	2	4	**6**	2	0	1	2
- moderate	0	1	0	**6***	0	0	0	1
- marked	0	0	0	**1**	0	0	0	1
Liver - Centrilobular hypertrophy (total)	0	1	**8****	**13****	0	2	5	**36****
- minimal	0	0	**1**	**2**	0	1	2	**3**

- mild	0	1	**6***	**11****	0	1	3	**33****
- moderate	0	0	**1**	**0**	0	0	0	**0**
Liver - Hepatocyte pigment centrilobular (total)	0	0	0	0	0	0	0	**3**
- minimal	0	0	0	0	0	0	0	1
- mild	0	0	0	0	0	0	0	1
- moderate	0	0	0	0	0	0	0	1
Liver - Hepatocyte vacuolation (total)	2	5	5	**9**	0	2	5	0
- minimal	0	0	2	3	0	0	3	0
- mild	2	2	1	5	0	2	2	0
- moderate	0	1	2	1	0	0	0	0
- marked	0	1	0	0	0	0	0	0
- severe	0	1	0	0	0	0	0	0
Kidney (Number of animals)	(52)	(52)	(52)	(52)	(52)	(52)	(52)	(52)
Kidney – pigment deposits, tubular cell	0	0	0	0	0	0	1	**5**
Kidney – tubular dilation	0	0	3	3	0	1	0	0
Thyroid (Number of animals)	(52)	(52)	(52)	(52)	(50)	(52)	(52)	(52)
Thyroid - Focal C-cell hyperplasia Total	11	3*	2*	5	3	5	4	**9**
Mammary Gland (Number of animals)	(52)	(52)	(52)	(52)	(50)	(52)	(52)	(52)
Mammary Gland - Lobular hyperplasia	0	0	2	0	12	13	10	**19**
Spleen (Number of animals)	(52)	(52)	(52)	(52)	(50)	(52)	(52)	(52)
Spleen - Pigmented macrophages	2	2	7	1	10	13	9	**18**
Sternum (Number of animals)	(52)	(52)	(52)	(52)	(50)	(52)	(52)	(52)
Sternum - Increased haematopoiesis	1	1	0	1	2	1	2	6
Stomach (Number of animals)	(52)	(52)	(52)	(52)	(50)	(52)	(52)	(52)
Stomach - Ulcer, focal, non-glandular stomach	2	0	0	0	0	1	**2**	**3**
Stomach - Ulcer, focal, glandular stomach	0	1	0	2	0	0	0	0

* Statistically significant difference from control group mean, p<0.05 (Fisher's Exact Test)

** Statistically significant difference from control group mean, p<0.01 (Fisher's Exact Test)

Neoplastic findings: There was a treatment-related increase in thyroid gland follicular cell adenomas in all treated groups of males. At 600 ppm, the tumours were above historical controls and statistically significant at the high-dose according to the Peto test.

There were slight increases in granular cell tumours in the brain of high-dose males and hepatocellular adenomas in high-dose females. Based on a range on 12 historical studies from between 2005 and 2009 performed at Charles River Edinburgh, both granular cell tumours in males and hepatocellular adenomas in females are above historical controls. The hepatocellular adenomas were slightly outside historical control range, while the granular cell tumours were moreso.

The study authors have included the increased incidence of adrenal cortical adenomas in control and low-dose females in their calculations as the incidences occurred above historical control for

the source animals. This change was not considered treatment-related as there was no dose-response and the control incidence was above the stated historical control range.

Table B.6.5.1/14: Intergroup comparison of select neoplastic lesions – carcinogenicity study[a]

	Dietary Concentration of SYN545192 (ppm)			
	0	**25**	**100**	**600**
Males				
Brain (Number of animals)	(52)	(52)	(52)	(52)
Granular cell tumour [B] - number	2	0	0	5
- % incidence	3.8			9.6
- Historical control range	0 – 6.0%			
Thyroid (Number of animals)	(52)	(52)	(52)	(52)
Follicular cell adenoma – number	1	4	5	**9***
- % incidence	2.0	7.6	9.6	**17.3**
- Historical control range	2 – 11%			
Females				
Adrenal Gland (Number of animals)	(51)	(51)	(51)	(52)
Cortical adenoma [B] – number	4	2	0	0
-% incidence	7.8	3.9		
- Historical control range	0 – 2%			
Liver (Number of animals)	(52)	(52)	(52)	(52)
Hepatocellular adenoma [B] – number	1	1	1	3
-% incidence	1.9	1.9	1.9	5.8
- Historical control range	0 – 5.5%			

[a] Data obtained from pages 50, 287 – 295 of the study report

* Statistically significant difference from control group mean, $p<0.05$ using Fisher's exact test

Table B.6.5.1/15: Peto analysis results for selected tumour types[a]

Organ	Tumour Type		P(1)	P(2)	P(3)	Direction of trend
Adrenal Gland	Cortical Adenoma	Females	0.005	0.035	0.64	-ve
Brain	Glandular Cell Tumour [B]	Males	0.17			+ve
Liver	Hepatocellular Adenoma	Females	0.36			+ve
Thyroid Gland	Follicular Cell Adenoma	Males	0.012	0.13		+ve

[a] Data obtained from pages 296 – 305 of the study report

(1) P-value for linear trend including groups 1 to 4

(2) P-value for linear trend including groups 1 to 3

(3) P-value for linear trend including groups 1 & 2

Investigator's Conclusion: "At 600/400 ppm, males and females had lower body weight gains, food consumption, and food utilisation. Clinical chemistry parameters of ALP, ALT and AST were consistently lower in both sexes, and covariate liver weight was higher in males after 104 weeks. A higher incidence of centrilobular hypertrophy in the livers of males and females was observed, and was accompanied by pigmented hepatocytes in females and hepatocyte vacuolation and hepatocellular eosinophilic foci in males. The incidence of thyroid follicular cell adenoma was higher in males at 600 ppm SYN545192.

At 100 ppm, there were considered to be no adverse treatment related findings. The only treatment related effects were a small decrease in food utilisation during the initial 4 weeks of the study in both sexes and centrilobular hypertrophy in males after 104 weeks of treatment. These findings were considered not to be adverse.

At 25 ppm, there were no treatment-related effects in this study.

Based on the results of this study, a No Observed Adverse Effect Level (NOAEL) was established at 100 ppm SYN545192, equating to 4.88 mg/kg/day in males and 6.66 mg/kg/day in females."

Reviewer's Conclusions:

Body weights and body weight gains were decreased at 600/400 ppm in males and females, respectively throughout the treatment period. Food consumption was decreased in males at 600 ppm at weeks 7 and 34 and in females at 400 ppm at weeks 5-9, 11-12, 16, 24, 28, 34-40, 44-46, 54-56, 64-66, 76, 80-96 and 100. Food utilization was decreased in weeks 1 – 4 in males at 600 ppm and at weeks 1 – 4, 9 – 13 and 1 – 13 in females at 400 ppm. Clinical signs of toxicity were limited to females at 400 ppm and were related to decreased condition with increases in hunched or held low body position, piloerection, staining on fur and thin appearance. Rolling gait was also noted in high-dose females. In the detailed clinical observations, there was a slight increase in high-dose males and females that did not react to the tactile stimulus and high-dose females reacted significantly more slowly to the tail flick stimulus.

In the liver, relative weights were increased in high-dose, carcinogencity phase males, along with increases in pale foci. Non-neoplastic, microscopic changes in toxicity-phase animals consisted of centrilobular hypertrophy in high-dose males and females and centrilobular hepatocyte pigmentation in high-dose females. In the carcinogenicity-phase animals, there was an increase in centrilobular hypertrophy at 600/400 ppm in males and females, an increase in eosinophilic cell foci and hepatocyte vacuolation at 600 ppm in males and an increase in hepatocyte centrilobular pigment at 400 ppm in females.

Other non-neoplastic changes in the carcinogenicity phase consisted of increased tubular cell pigment deposits in the kidneys, focal c-cell hyperplasia of the thyroid, lobular hyperplasia of the mammary glands and pigmented macrophages in the spleen of high-dose females.

There was a treatment-related increase in follicular cell adenomas of the thyroid in males. At 600 ppm, the increase was statistically significant and above historical controls.

The LOAEL is 600/400 ppm (30.17/27.44 mg/kg bw per day) in males and females respectively, based on decreased body weights, body weight gains, food consumption, food utilization, lack of tactile stimulus and liver changes in males and females, thyroid follicular cell adenomas in males and hunched or held low body position, piloerection, staining on fur and thin appearance, slow reaction to tail flick and rolling gait, increased tubular cell pigment deposits in the kidneys, focal c-cell hyperplasia of the thyroid, lobular hyperplasia of the mammary glands and pigmented macrophages in the spleen in females. The NOAEL is 100 ppm (4.88/6.66 mg/kg bw per day).

B.6.5.2 Carcinogenicity study in the mouse

Report:	IIA 5.5.3/01. Mackay C, 2012b. SYN545192 - 80 week mouse dietary carcinogenicity study. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Laboratory Report No. 32209, 06 February 2012. Unpublished. (Syngenta File No.SYN545192_10189). EPA MRID No. 48604448

GUIDELINES: Carcinogenicity – Mouse (feeding) OECD 451 (2009): OPPTS 870.4200 (1998): 88/302/EEC B.32 (1988): JMAFF No.12-Nousan-8147 (2000)

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

Dose Level Selection and Dose Adequacy:

Dose levels for this study were based upon an evaluation of preliminary studies carried out at Charles River Edinburgh (SYN545192 - 13 Week Dietary Toxicity Study in Mice, Charles River Study No. 459554, Report Number: 30511 and SYN545192 - 28 Day Mouse Dietary Toxicity Study, Charles River Study No. 459376, Report Number: 30293). In the 28 day study there was initial body weight loss at 500 ppm and statistically significant lower mean body weights from Day 2 until the end of treatment in both sexes. In males at 300 ppm there was initial body weight loss and statistically significant lower mean body weight from Day 2 until Day 5. In the 13 week study there were significant initial body weight losses in animals dosed at 500 ppm, resulting in the termination of two males in the toxicokinetic satellite group. At 300 ppm there was a significant decrease in body weight gain over the 90 day treatment period. Based on the findings of the previous studies, dose levels of 0, 20, 60 and 200 ppm were chosen for this study, which took into account the maximum tolerated dose in the test model.

The selection of dose levels for this 80-week study in mice was made following consultation with a Dose Adequacy Review Team (DART) of the US Environmental Protection Agency. In the DART meeting on September 29, 2009, Syngenta presented the results of the 28-day and 90-day mouse studies and its proposal to select dose levels of 0, 20, 60 and 200 ppm. The EPA DART felt that a top dose of 200 or 250 ppm may not be adequate to cause toxicity and assess the carcinogenic potential of SYN545192 in mice, and recommended that a top dose of 300 ppm be selected, with a mid-dose level of 100 ppm. [1] Syngenta expressed concern that at 500 ppm, excessive body weight gain decrements led to early termination of two males at this dose in the 90-day study, and therefore, the highest dose in an 80-week study should be at least 2-fold lower than this excessive dose. While recognizing the DART's observation that body weight effects at 300 ppm were less noticeable in the 28-day study than in the 90-day study, Syngenta expressed that such variability can often occur in shorter-term studies, particularly when a smaller number

[1] Kidwell, J. (2010). SYN545192 (HAMBRA): Report of the Dose Adequacy Review Team (DART) – Concurrence with Dose Selection for the 80 Week Mouse Study. US Environmental Protection Agency. DP Barcode D371740. February 2, 2010.

of animals are tested (e.g. 5/sex/group in the 28-day study compared to 10/sex/group in the 90-day study)

After careful consideration of all inputs including the position of the EPA DART, Syngenta selected dose levels of 0, 20, 60 and 200 ppm for this study. The results of this study confirm that treatment-related, systemic toxicity was expressed at the top dose of 200 ppm that was consistent with findings in the earlier 90-day mouse study. Male mice treated at 200 ppm had statistically significantly lower group mean body weights during the first 7 weeks of the study. In addition, a higher incidence of simple mucosal hyperplasia in the large intestine (colon and caecum) was observed at 200 ppm in both males and females. Considering that SYN545192 at 200 ppm produced long-term systemic toxicity in male and female mice, this study fulfilled Guideline requirements for an adequate evaluation of carcinogenicity.

EXECUTIVE SUMMARY

In a carcinogenicity study with SYN545192 (purity 97%), four groups of 50 CD-1 mice/sex/group were fed diets containing 0, 20, 60 or 200 ppm SYN545192 for at least 80 consecutive weeks.

The animals were monitored regularly for viability and for signs of ill health or reaction to treatment. Body weights and food consumption were measured and recorded at pre-determined intervals from pre-trial up until the completion of treatment. Blood samples for haematology were also collected from all surviving animals prior to terminal kill at week 80. Blood films were made from all surviving animals during week 52/53.

All surviving animals were terminated after the completion of 80 weeks of treatment and underwent a detailed necropsy examination with a comprehensive histological evaluation.

There were no effects on survival, body weight, body weight gain, food or water consumption, haematology, organ weights or gross pathology.

Females dosed at 200 ppm exhibited increased incidences of piloerection and rolling gait. Males and females at 200 ppm exhibited increased mucosal hyperplasia of the colon and caecum.

This study did not reach the maximum tolerated dose (MTD.)

The LOAEL was 200 ppm (26.18 mg/kg bw per day in males and 29.26 mg/kg bw per day in females) based on hyperplasia of the colon and caecum in males and females and piloerection and rolling gait in females. The NOAEL was 60 ppm (7.55 mg/kg bw per day in males and 8.67 mg/kg bw per day in females).

This carcinogenicity study in the mouse is unacceptable and does not satisfy the guideline requirement for a carcinogenicity study (OPPTS 870.4200); OECD 451 in mice.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545192
Description:	Technical, beige powder
Lot/Batch number:	SMU9BP005
Purity:	97% a.i
CAS#:	Not reported
Stability of test compound:	Reanalysis date end February 2013 (stored <30°C)

Vehicle and/or positive control: The test substance was administered in Rat and Mouse (Modified) No.1 (RM1) Diet SQC Expanded (Fine Ground).

Test Animals:

Species	Mouse
Strain	CD-1 (Crl:CD-1(ICR))
Age/weight at dosing	Approximately 7 weeks / 28.8-41.7 g (males), 22.7-33.9 g (females)
Source	Charles River UK Limited, Margate, Kent, UK
Housing	Male animals were housed individually and female animals were housed up to 3 per cage in suspended polypropylene cages (overall dimensions 48 x 15 x 13 cm) with stainless steel grid tops and solid bottoms containing a separate stainless steel food hopper
Acclimatisation period	23 days
Diet	Rat and Mouse (modified) No. 1 Diet SQC Expanded (supplied by Special Diets Services Limited, 1 Stepfield, Witham, Essex, UK) *ad libitum*
Water	Domestic water *ad libitum*
Environmental conditions	Temperature: 16-24°C Humidity: 14-67% Air changes: Minimum of 15/hour Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

In-life dates: Start: 01 October 2009 End: 17 August 2011

Animal assignment: On arrival from the suppliers, the animals were introduced to cages on racks. Cages were racked by treatment group and vertically throughout the rack. Each month from the commencement of pre-trial, each column of cages on a rack was moved one position to the right. These changes were carried out to minimize environmental effects. During pre-trial, body weights were checked to ensure that all groups had a similar group mean body weight for each sex and all were found to be within the 20% limit of variation.

Table B.6.5.2/01: Study design

Test group	Dietary concentration (ppm)	Males	Females
Control	0	1-50	201-250
Low	20	51-100	251-300

Mid	60	101-150	301-350
High	200	151-200	351-400

Diet preparation and analysis: A 200 g stock was prepared by mixing the test substance with the required amount of untreated control diet in an automated mortar and pestle until visibly homogeneous. A high level premix at 5000 ppm was prepared by adding the appropriate amount of blank RM1 diet to the 200 g stock and mixing. Diet formulations for the high dose group were prepared as a dilution from the high level premix. A low level premix at 500 ppm was prepared as a dilution from the high level premix. Diet formulations for the intermediate and low level dose groups were prepared as a dilution from the low level premix. Blank diet (without the test substance) was prepared for control animals. Diet formulations were prepared at appropriate intervals (typically every 2 weeks) for administration to the animals.

The diet formulations were analysed for achieved concentration and homogeneity at intervals throughout the study.

Analysis results: For diets prepared for use in Weeks 1, 13, 25, 39, 51, 65 and 79, the mean test substance concentration at each level was between -8.5% and 5.3% of the nominal concentration, with a maximum variability between replicate samples of 4.0%, indicating that the diets were correctly and homogeneously prepared. SYN545192 was not detected in control diet at any timepoint.

Stability results: Stability data for diets at 10-5000 ppm was confirmed for 30 days when stored at ambient temperature in the dark, following work carried out in the Analytical Chemistry Services of Charles River, Edinburgh under a separate protocol.

Observations: All animals were checked early morning and as late as possible each day for signs of viability. Once each week all animals received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.

Body weight: Body weights were recorded twice each week commencing during the pre-trial period until week 4 of treatment, then once weekly from week 5 until week 14 and approximately once every 2 weeks from week 16 until the end of treatment.

Food consumption and test substance intake: The quantity of food consumed by each cage of animals was measured and recorded twice each week commencing during the pre-trial period until week 4 of treatment, then once weekly from week 5 until week 14 and once every 2 weeks from week 16 until the end of treatment.

Food utilisation was calculated for weeks 1-4, 5-8, 9-13 and 1-13 by dividing (cage mean weight gain x 100) by cage total food consumption

The amount of test substance ingested was calculated at regular intervals during treatment using the following formula:

Achieved intake (mg/kg/day) = Nominal Concentration (ppm) x Food Consumption (g/day)

Mid-point Body Weight (g)

Water consumption: Qualitatively monitored by visual inspection of the water bottles on a weekly basis throughout the study.

Haematology: A blood film smear was made from all surviving animals at Week 52/53 and stained for possible examination.

Blood samples (as much blood as possible) were obtained from all surviving animals via the orbital sinus under isoflurane anaesthesia and transferred into tubes containing EDTA on the day of terminal kill. The animals were not deprived of food overnight prior to sampling.

The blood was transferred into tubes containing EDTA and assayed for:

White blood cell count
Differential white blood cell count:
Neutrophils
Lymphocytes
Monocytes
Eosinophils
Basophils
Large Unclassified Cells

(For four animals, a manual differential count was necessary; therefore no large unclassified cells counts are available for these animals).

A blood film smear was also made from all EDTA haematology samples at week 80 and stained for possible examination.

Femoral bone marrow smears were taken at necropsy and stored for possible evaluation.

Investigations post-mortem*:*

Macroscopic examination: All animals were examined post mortem. This involved an external observation and an internal examination of all organs and structures.

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands
brain
epididymides
heart
kidneys
liver and gall bladder
ovaries
spleen
testes
uterus

Paired organs were weighed separately and the sum used for reporting purposes.

Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative:

abnormal tissue (including local lymph nodes to masses)
adrenal gland
aortic arch
brain (forebrain, midbrain, cerebellum, pons)
bone marrow (sternum)
caecum
colon
duodenum
epididymis
eyes
femoral bone (including stifle joint)
Harderian gland
heart
ileum
jejunum
kidney
larynx
liver and gall bladder
lung
nasal cavity
lymph node – mesenteric
mammary gland
nerve – sciatic
optic nerve
lymph node - submandibular
oesophagus
ovary
pancreas
parathyroid gland
pharynx
pituitary gland
prostate gland
rectum
sub maxillary salivary gland
seminal vesicle
spinal cord (cervical, midthoracic, lumbar)
skin
spleen
sternum (including bone marrow)
stomach
testis
thymus
thyroid gland
tongue
trachea
urinary bladder
uterus
vagina
voluntary muscle (thigh)

Microscopic examination: All processed tissues were examined by light microscopy.

From all animals, a sample of liver (*ca* 4 mm) was taken from the left lateral lobe, divided in two and snap frozen in liquid nitrogen in individual RNA-ase free tubes. All samples were taken as quickly as possible and stored at *ca* -70°C pending possible future analysis. The remainder of the liver, after sampling for histopathology, was sectioned into 7-8 pieces and snap frozen in liquid nitrogen and stored at *ca* -70°C pending possible future analysis.

In addition to the gastro-intestinal tract sections obtained for histological examination, two tissue sections (both *ca* 10 mm in length) were taken from each segment of the gastro-intestinal tract (stomach, duodenum, jejunum, ileum, caecum, colon and rectum) as soon as possible after post-mortem. Each 10 mm section taken was snap frozen in liquid nitrogen and placed in an RNAase-free tube prior to storage at *ca* -70°C pending possible future analysis.

Statistics: Body weight, cumulative body weight gain, food consumption, food utilisation, haematology, and organ weight data were analysed using a parametric ANOVA and pairwise

comparisons made using the Dunnett's t-test. The following pairwise comparisons were performed: Control Group vs Low Dose, Control Group vs Intermediate Dose and Control Group vs High Dose. Organ weights were also analysed by analysis of covariance (ANCOVA) using terminal kill body weight as covariate followed by a pairwise comparison using Dunnett's t-test. Analyses of variance and covariance were carried out using the MIXED procedure in SAS. Kaplan-Meier survival estimates were calculated separately for each sex and treatment group. Histological incidence data were analysed using Fisher's Exact Probability Test. Pair-wise comparisons of the incidence of tumour and histological lesions was made using Fisher's Exact Probability test function within PLACES 2000. Further analysis was performed using Peto's time adjusted methods. Methods used for the age-adjusted analysis of fatal and non-fatal tumours were based on the IARC guidelines.

RESULTS AND DISCUSSION

Mortality: There was no effect on mortality.

Clinical observations: There was a slight increase in the incidence of piloerection and rolling gait in females. The two clinical signs were seen in the same animals at the same time in 40% of animals exhibiting either symptom. At lower doses, comorbidity was seen in 21-23% of affected animals.

Table B.6.5.2/02: Select clinical signs[a]

	Dietary concentration of SYN545192 (ppm)							
Group	**Males**				**Females**			
	0	**20**	**60**	**200**	**0**	**20**	**60**	**200**
(no of animals)	**(50)**	**(50)**	**(50)**	**(50)**	**(50)**	**(50)**	**(50)**	**(50)**
Piloerection	8	8	4	9	9	7	9	**16**
Rolling gait	3	6	1	3	8	9	7	**12**
[a] Data obtained from page 104 of the study report								
Bolded values determined to be treatment-related								

Bodyweight and weight gain: Absolute body weights were not affected by treatment. In males, there was a statistically significant decrease in body weights in the 200 ppm dose group in the first 7 weeks of treatment; however, body weights were also decreased in the 20 ppm dose group over the same time period to a greater extent than the 200 ppm dose group and the decreased body weights only reached the threshold for adverse effects in the first half of the first week of treatment. In females, body weights were decreased to a statistically significant extent in all dose groups sporadically through the first 12 weeks of treatment. There was no dose response and the changes were not considered related to treatment. See Table B.6.5.2/03.

Body weight gains were likewise unaffected by treatment. There were statistically significant increases and decreased compared to controls in all dose-group, but no consistent dose-response.

Table B.6.5.2/03: Intergroup comparison of bodyweight (g) - selected weeks[a]

	Dietary concentration of SYN545192 (ppm)							
	Males				Females			
Weeks	**0**	**20**	**60**	**200**	**0**	**20**	**60**	**200**
-1	33.7 ± 2.1	33.2 ± 2.3	33.5 ± 1.8	33.4 ± 2.2	26.9 ± 2.1	26.0 ± 1.7* (↓3)	25.6 ± 2.0** (↓5)	25.7 ± 1.8** (↓4)
-1	35.0 ± 2.2	34.3 ± 2.5 (↓2)	33.9 ± 1.9* (↓3)	33.7 ± 2.4* (↓4)	27.4 ± 2.3	26.8 ± 2.0	26.2 ± 2.1* (↓4)	26.0 ± 1.9** (↓5)
0	36.1 ± 2.4	35.5 ± 2.6	35.5 ± 2.0	35.2 ± 2.5	28.3 ± 2.5	27.3 ± 1.8* (↓4)	27.2 ± 2.0* (↓4)	27.0 ± 2.0** (↓5)
1	36.5 ± 2.4	35.8 ± 2.8	36.0 ± 2.1	34.6 ± 2.6** (↓5)	28.1 ± 2.1	27.0 ± 1.9* (↓4)	27.5 ± 2.2 (↓2)	27.1 ± 2.1* (↓4)
2	38.0 ± 2.6	36.6 ± 3.0* (↓4)	37.2 ± 2.4 (↑2)	36.8 ± 2.9 (↑3)	29.0 ± 2.4	27.7 ± 2.3* (↓5)	28.2 ± 2.4 (↓3)	27.9 ± 2.1* (↓4)
5	41.1 ± 3.2	38.9 ± 3.6** (↓5)	40.1 ± 3.0 (↓2)	39.5 ± 3.6* (↓4)	32.0 ± 3.5	30.0 ± 2.9** (↓6)	30.8 ± 2.5 (↓4)	31.1 ± 2.9 (↓3)
10	44.9 ± 4.3	44.4 ± 4.7	45.1 ± 4.5	44.3 ± 4.7	35.5 ± 4.8	33.2 ± 4.2* (↓7)	34.9 ± 3.9 (↓2)	34.6 ± 4.4 (↓3)
16	49.0 .4	48.3 ± 5.3	48.3 ± 5.5	47.9 ± 5.7	39.9 ±7 .4	37.0 ± 5.7 (↓7)	37.5 ±6 .1 (↓6)	38.2 ± 6.2 (↓4)
20	51.9 ± 6.1	51.1 ± 5.7	50.5 ± 6.4	50.3 ± 6.0	43.4 ± 7.6	40.8 ± 7.2 (↓6)	40.8 ± 7.6 (↓6)	40.9 ± 7.7 (↓6)
30	53.2 ± 6.6	53.8 ± 6.2	52.9 ± 7.3	52.7 ± 6.2	46.2 ± 8.8	44.6 ± 9.0	44.4 ± 8.5	45.0 ± 8.4
40	55.3 ± 6.7	55.5 ± 6.4	54.0 ± 7.0	54.5 ± 6.5	49.0 ± 9.2	48.6 ± 11.1	47.3 ± 9.2	48.5 ± 9.4
50	56.9 ± 6.9	56.9 ± 6.1	55.5 ±7 .9	56.2 ± 6.8	52.2 ± 11.1	49.7 ± 93.7 (↓5)	49.4 ± 10.1 (↓5)	50.9 ± 10.1 (↓2)
60	57.5 ± 6.8	57.2 ± 6.8	56.0 ± 7.5	56.1 ± 7.2	53.1 ± 11.4	50.9 ± 10.2	50.4 ± 11.0	51.7 ± 9.3
62	56.7 ± 6.9	56.4 ± 6.8 (↓1)	55.3 ± 7.5 (↓2)	55.3 ± 7.3 (↓2)	53.2 ± 11.3	50.4 ± 9.9 (↓5)	50.8 ± 10.8 (↓5)	50.7 ± 10.0 (↓5)
70	57.1 ± 7.0	57.7 ± 7.7	56.8 ± 7.6	57.2 ± 6.6	53.8 ± 12.4	52.8 ± 9.9 (↓2)	52.5 ± 10.9 (↓2)	52.3 ± 10.8 (↓3)
80	56.4 ± 7.0	58.0 ± 8.2	56.4 ± 7.4	56.2 ± 6.3	55.4 ± 12.2	52.2 ± 9.9 (↓6)	53.8 ± 11.7 (↓3)	51.4 ± 10.8 (↓7)

[a] Data obtained from pages 107 – 116 of the study report

* Statistically significant difference from control group mean, p<0.05; ** Statistically significant difference from control group mean, p<0.01

Table B.6.5.2/04: Intergroup comparison of bodyweight gain (g) - selected weeks[a]

	Dietary concentration of SYN545192 (ppm)							
	Males				Females			
Weeks	**0**	**20**	**60**	**200**	**0**	**20**	**60**	**200**
0-1	1.0 ± 0.5	0.8 ± 0.7 (↓20)	1.0 ± 6 (0)	0.5 ± 0.9** (↓50)	-0.6 ± 1.6	-0.5 ± 1.0 (↑17)	0.6 ± 1.2** (↑200)	0.3 ± 1.0** (↑150)
0-2	1.9 ± 0.7	1.2 ± 1.0** (↓37)	1.7 ± 1.0 (↓10)	1.6 ± 0.9 (↓16)	1.3 ± 1.5	0.6 ± 1.3* (↓54)	0.9 ± 1.4 (↓31)	1.2 ± 0.9 (↓8)
0-3	3.4 ± 1.0	2.0 ± 1.5** (↓41)	2.7 ± 1.2* (↓21)	2.6 ± 1.2** (↓24)	1.8 ± 1.9	1.4 ± 1.5 (↓22)	1.7 ± 1.3 (↓6)	1.9 ± 1.5 (↑6)
0-4	4.4 ± 1.2	2.1 ± 1.7** (↓52)	3.9 ± 1.4 (↓11)	3.5 ± 1.4** (↓20)	2.1 ± 1.9	1.5 ± 1.5 (↓29)	3.0 ± 1.7* (↑43)	3.3 ± 1.6** (↑57)
0-5	5.1 ± 1.6	3.4 ± 2.0** (↓33)	4.6 ± 1.6 (↓10)	4.2 ± 1.6 (↓18)	3.7 ± 2.4	2.7 ± 2.0* (↓27)	3.6 ± 1.7 (↓3)	4.2 ± 1.8 (↑14)
0-10	8.9 ± 3.1	8.9 ± 3.1 (0)	9.6 ± 3.3 (↑8)	9.1 ± 2.9 (↑2)	7.1 ± 3.8	6.0 ± 3.1 (↓15)	7.7 ± 3.3 (↑8)	7.7 ± 3.3 (↑8)
0-13	11.4 3.9	10.9 ± 3.6 (↓4)	11.1 ± 4.1 (↓3)	10.7 ± 3.6 (↓6)	8.5 ± 4.5	7.5 ± 4.2 (↓12)	9.0 ± 4.4 (↑6)	8.7 ± 4.4 (↑2)
0-26	17.1 ± 5.7	17.5 ± 4.9 (↑2)	17.1 ± 6.3 (0)	16.5 ± 4.6 (↓4)	15.8 ± 7.5	16.3 ± 7.5 (↓3)	16.9 ± 7.8 (↑7)	16.9 ± 7.3 (↑7)
0-52	20.2 ± 5.8	21.5 ± 5.2 (↑6)	19.7 ± 6.6 (↓2)	20.3 ± 5.8 (0)	23.3 ± 10.5	22.1 ± 8.8 (↓5)	22.5 ± 9.6 (↓3)	24.1 ± 9.7 (↑3)
0-80	20.2 ± 5.9	22.4 ± 7.2 (↑10)	20.9 ± 6.5 (↑3)	20.8 ± 5.3 (↑3)	26.9 ± 11.3	25.1 ± 9.4 (↓7)	26.9 ± 10.8 (0)	24.8 ± 10.1 (↓8)

[a] Data obtained from pages 117 – 124 of the study report

* Statistically significant difference from control group mean, $p<0.05$; ** Statistically significant difference from control group mean, $p<0.01$

Food consumption and compound intake: There were no effects on food consumption or food utilisation.

Achieved dose levels study were calculated and mean values are shown below:

Table B.6.5.2/05: Mean dose received (mg/kg/day)

SYN545192 (ppm)	20	60	200
Males	2.62	7.55	26.18
Females	2.89	8.67	29.26

Water consumption: There were no treatment-related effects on water consumption.

Haematology: In mid- and high-dose females, there was an apparent increase in WBC and neutrophil counts (see Table II 5.5.3/06). While the change was statistically significant in the high-dose female neutrophil counts, there was a large intra-group variability and no effects on the associated organs. The change was considered unrelated to treatment.

Table B.6.5.2/06: Intergroup comparison of select haematological parameters[a]

	Dietary concentration of SYN545192 (ppm) Females			
Parameter	**0**	**20**	**60**	**200**
WBC	5.17 ± 2.04	5.84 ± 5.76	6.62 ± 5.25 (↑28)	7.27 ± 5.45 (↑41)
Neutrophils	1.17 ± 0.53	1.14 ± 0.72	1.73 ± 1.46 (↑48)	2.92 ± 3.12** (↑150)
[a] Data obtained from page 138 of the study report				
* Statistically significant difference from control group mean, p<0.05; ** Statistically significant difference from control group mean, p<0.01				

Sacrifice and pathology:

Organ weights: Adrenal weights were increased compared to concurrent controls in the 60 and 200 ppm male mice; however, as there was no change in gross or histological findings, the change was not considered treatment-related or adverse. Likewise, the increased epididymide weights were not considered treatment-related or adverse. In males, spleen weights were decreased at 60 ppm and increased at 200 ppm in males and decreased at 200 ppm in females. Due to lack of dose response in males and lack of associated haematological or histopathological changes, the organ weight findings were not considered treatment-related or adverse.

Table B.6.5.2/07: Intergroup comparison of select organ weights (g)[a]

	Dietary concentration of SYN545192 (ppm) Males			
Parameter	**0**	**20**	**60**	**200**
Body weight	55 ± 7	57 ± 8	55 ± 7	55 ± 6
Adrenals	0.0042 ± 0.0018	0.0045 ± 0.0020	0.0053 ± 0.0027 (↑26)	0.0071 ± 0.0132 (↑69)
Rel. Adrenals	0.00763 ± 0.00323	0.00825 ± 0.00431	0.00947 ± 0.00445 (↑29)	0.01310 ± 0.02364 (↑78)
Epididymides	0.1357 ± 0.0207	0.1520 ± 0.1027	0.1348 ± 0.0153	0.1720 ± 0.2615 (↑27)
Rel. Epididymides	0.24970 ± 0.04366	0.27007 ± 0.15648	0.24799 ± 0.03910	0.30142 ± 0.39052 (↑21)
Spleen	0.138 ± 0.078	0.128 ± 0.056	0.116 ± 0.050 (↓16)	0.179 ± 0.186 (↑30)
Rel. Spleen	0.2534 ± 0.1466	0.2266 ± 0.0910	0.2117 ± 0.0873 (↓16)	0.3220 ± 0.3209 (↑27)
	Females			

Parameter	0	20	60	200
Body weight	54 ± 12	52 ± 11	51 ± 11	51 ± 10
Spleen	0.279 ± 0.378	0.279 ± 0.296	0.271 ± 0.286	0.214 ± 0.154 (↓23)
Rel. Spleen	0.53315 ± 0.6722	0.5828 ± 0.6525	0.5725 ± 0.6715	0.4428 ± 0.3306 (↓17)
[a] Data obtained from pages 139, 141, 142 and 144 of the study report				
* Statistically significant difference from control group mean, p<0.05; ** Statistically significant difference from control group mean, p<0.01				

Macroscopic findings: There was a slight increase in pale livers in high-dose females. As this was not correlated any changes in organ weights or clinical chemistry, the change was not considered adverse. There was an increase in lymph node enlargement in males at doses ≥ 60 ppm; however, this was not correlated with organ weight or histopathological changes and was not seen in females. The change was not considered treatment-related or adverse.

Table B.6.5.2/08: Intergroup comparison of select necropsy findings[a]

	Dietary concentration of SYN545192 (ppm)							
Group	**Males**				**Females**			
	0	**20**	**60**	**200**	**0**	**20**	**60**	**200**
(no of animals)	**(50)**	**(50)**	**(50)**	**(50)**	**(50)**	**(50)**	**(50)**	**(50)**
Liver								
Pale	1	2	2	1	1	1	3	**7**
Lymph node (mesenteric)								
Englargement	0	0	3	6	8	5	6	5
[a] Data obtained from page 153 – 156 of the study report								
Bolded values determined to be treatment-related								

Microscopic findings:

Non-neoplastic findings: Non-neoplastic findings were limited to the colon and caecum of the large intestine of both sexes (see Table B.6.5.2/09). As seen in tables B.6.5.2/10 and B.6.5.2/11, hyperplasia in the two organs was increased in incidence and severity at the high dose. There were sporadic incidences at doses ≤ 60 ppm; however, these incidences were considered spontaneous and not treatment-related.

Table B.6.5.2/09: Intergroup comparison of select non-neoplastic histopathological findings[a]

	Dietary concentration of SYN545192 (ppm)							
Group	**Males**				**Females**			
	0	**20**	**60**	**200**	**0**	**20**	**60**	**200**
Adrenal gland (# examined)	49	50	49	50	50	50	50	49
No abnormality detected	19	16	17	18	8	9	7	1*
Subcapsular cell	19	24	18	27	37	40	40	44

hyperplasia								
Cortical atrophy	3	1	1	5	0	0	0	0
Extramedullary haematopoiesis	0	0	0	0	1	0	0	3
Caecum (# examined)	49	50	49	50	48	49	49	48
No abnormality detected	48	49	48	42*	43	45	45	39
Hyperplasia, mucosal, simple	0	0	1	**4**	0	0	0	**2**
Oedema, submucosal	0	1	0	0	0	0	1	**3**
Colon (# examined)	49	50	50	49	48	49	49	48
No abnormality detected	47	47	50	36**	45	45	48	36*
Hyperplasia, mucosal, simple	1	2	0	**13****	0	0	0	**10****
Epididymis (# examined)	50	50	50	50				
No abnormality detected	41	27**	34	31*				
Absence of sperm	5	8	12	10				
Liver (# examined)	50	50	50	50	50	50	50	50
Pigmented macrophages	1	0	0	0	5	2	2	9
Lungs (# examined)	50	50	50	50	50	50	50	50
Inflammation/ inflammatory cell infiltration, peribronchial/ peribronchiolar	1	4	5	1	2	0	2	6
Lymph node (**mesenteric**; # examined)	49	50	49	50	50	50	50	47
Pigmented macrophages	7	5	6	5	11	12	8	16
Spleen (# examined)	50	50	50	50	50	50	50	49
Extramedullary haematopoiesis	45	46	47	46	34	37	39	40
*p<0.05, **p<0.01								
[a] Data obtained from pages 170 – 210 of the study report								

Table B.6.5.2/10: Intergroup comparison of the incidence of simple mucosal hyperplasia in the colon[a]

	Dietary concentration of SYN545192 (ppm)							
Group	**Males**				**Females**			
	0	**20**	**60**	**200**	**0**	**20**	**60**	**200**
(no of animals)	**(49)**	**(50)**	**(50)**	**(49)**	**(48)**	**(49)**	**(49)**	**(48)**
Minimal	0	1	0	3	0	0	0	**3**
Mild	1	0	0	**6**	0	0	0	**6***
Moderate	0	1	0	**3**	0	0	0	**1**
Marked	0	0	0	**1**	0	0	0	0
Total Incidence	1	2	0	**13****	0	0	0	**10****
*p<0.05, **p<0.01								
[a] Data obtained from page 32 of the study report								

Table B.6.5.2/11: Intergroup comparison of the incidence of simple mucosal hyperplasia in the caecum[a]

	Dietary concentration of SYN545192 (ppm)							
Group	**Males**				**Females**			
	0	**20**	**60**	**200**	**0**	**20**	**60**	**200**
(no of animals)	**(49)**	**(50)**	**(49)**	**(50)**	**(48)**	**(49)**	**(49)**	**(48)**
Minimal	0	0	0	**1**	0	0	0	0
Mild	0	0	1	**3**	0	0	0	**2**
Total Incidence	0	0	1	**4**	0	0	0	**2**
[a] Data obtained from page 32 of the study report								

Neoplastic findings: Adenomas of the Harderian glands occur at incidences above or at the top end of historical controls in all treated animals (see Table B.6.5.2/12). However, there was a lack of dose-response with low- and high-dose groups showing higher incidences than the mid-dose group in both males and females. While the incidences are outside historical control, due to the lack of dose-response and lack of correlated organ in humans, the relevancy of this finding is unknown.

Table B.6.5.2/12: Intergroup comparison of the incidence of adenoma and adenocarcinoma of the Harderian gland

	Dietary concentration of SYN545192 (ppm)							
Group	**Males**				**Females**			
(no of animals)	**(50)**	**(50)**	**(50)**	**(50)**	**(50)**	**(50)**	**(50)**	**(50)**
	0	**20**	**60**	**200**	**0**	**20**	**60**	**200**
Harderian gland (# examined)	50	50	50	50	50	50	50	50
Adenocarcinoma [M]	0	1	0	0	0	0	0	0
Adenoma [B]	2	7 (14%)	4 (8%)	8 (16%)	0	4 (8%)	3 (6%)	5 (10%)
Adenoma HC[b]	2 – 4 (4 – 8%)				1 – 2 (2 – 4%)			
Ovary (# examined)	-	-	-	-	50	50	50	50

Tubulostromal adenoma [B]					0	1	1	2
Sex cord/Stromal tumour [B]					0	0	0	1
Uterus (# examined)	-	-	-	-	50	50	50	50
Leiomyosarcoma [M]					0	0	2	0
Leiomyoma [B]					1	1	1	3
*p<0.05, **p<0.01								
[a] Data obtained from pages 211 – 216 of the study report								
[b] HC – Historical controls consist of 4 studies performed in 2007 with 49 – 50 animals examined per study (from page 33 of the study report)								

Study Author's Conclusions: "Lower mean body weight and lower body weight gain were observed in males at 200 ppm during the initial weeks of the study and are considered to be related to treatment.

The higher incidence of simple mucosal hyperplasia in the large intestine in both sexes at 200 ppm was considered to be treatment related and to be clear evidence of systemic toxicity at this dose. There was no effect on the large intestine at either 20 or 60 ppm in either sex.

It is considered that the higher incidence of Harderian gland adenomas in treated mice was not treatment related.

CONCLUSION: In conclusion, dietary administration of SYN545192 to mice at 0, 20, 60 and 200 ppm, for a period of up to 80 weeks was well tolerated. There were no effects on survival and no clinical signs of toxicity.

Mean body weight and body weight gain were lower than control in males at 200 ppm in the initial weeks of the study.

The administration of SYN545192 at 200 ppm was associated with a higher incidence of simple mucosal hyperplasia in the large intestine in both sexes. There was no effect on the large intestine at either 20 or 60 ppm in either sex.

There were no treatment-related increases in neoplastic findings.

Based on the findings in this study, a NOEL is established at 60 ppm, which is equivalent to 7.55 mg/kg/day in males and 8.67 mg/kg/day in females."

Reviewer's Conclusions: The reviewer agrees with the study author's conclusions that there is no effect on survival, food consumption, water consumption, haematology, organ weights or gross pathology.

However, the reviewer does not agree that there were effects on body weight or body weight gain in either sex. Changes were transient and not of sufficient magnitude to be considered adverse.

Clinical signs of toxicity were apparent in 200 ppm females with increased incidences of piloerection and rolling gait.

Histopathological changes were limited to hyperplasia or the colon and caecum in males and females at 200 ppm.

There was no evidence of neoplastic changes in either sex at the doses tested.

The LOAEL was 200 ppm (26.18 mg/kg bw per day in males and 29.26 mg/kg bw per day in females) based on hyperplasia of the colon and caecum in males and females and piloerection and rolling gait in females. The NOAEL was 60 ppm (7.55 mg/kg bw per day in males and 8.67 mg/kg bw per day in females).

Deficiencies: This study did not reach MTD.

Report:	IIA 5.6.1/02 Adamska M (2012). **SYN545192 – Two-Generation Reproduction Toxicity Study in the Han Wistar Rat,** Harlan Laboratories Ltd. Wölferstrasse 4, 4414 Füllinsdorf, Switzerland. Laboratory Report No. C93200, issue date 14 March 2012. Unpublished. MRID #48604449

Guidelines: OECD 416 (2001): OPPTS 870.3800 (1998): Ministry of Agriculture, Forestry and Fisheries, Japan, 12 – NohSan No.8147 (2000).

Sponsor: Syngenta Crop Protection, LLC 410 Swing Road Post Office Box 18300 Greensboro, NC 27419-8300 USA

Executive Summary: Four groups of HanRcc:WIST(SPF) rats (P generation) received the test article, SYN545192, in the diet for 10 weeks and were then paired (one male with one female) for mating. The F1 generation animals were selected from the weaned F1 litters. The F1 parents were maintained on test diets for at least 90 days and then were paired for mating. The F2 offspring were sacrificed at weaning. Each group consisted of 25 male and 25 female rats. SYN545192 was administered orally, by ingestion, continuously throughout the study at dietary levels:

0, 25, 100, 600 ppm in Males-
Parental: 0, 1.7, 6.8, 40.5 mg/kg/day;
F1: 0, 1.9, 7.8, 48.0 mg/kg/day

0, 25, 100, 250 ppm in Females-
Parental: 0, 2.0, 8.2, 19.4 mg/kg/day;
F1: 0, 2.1, 8.7, 22.0 mg/kg/day

All dams and remaining pups were sacrificed on day 21 p.p. (post partum) and males were sacrificed when they were no longer needed for reproduction.

The parental NOAEL (No Observed Adverse Effect Level) was 100 ppm (equivalent to 6.8 mg/kg/day for P generation males and 8.2 mg/kg/day during pre-pairing). The parental LOAEL is 600 ppm in males (40.5 mg/kg/day) and 250 ppm in females (19.4 mg/kg/day) due to decreased body weight, decreased body weight gain, and decreased food consumption.

The offspring NOAEL for general toxic effects was 100 ppm (equivalent to 7.8 mg/kg/day for F1 generation males and 8.7 mg/kg/day during pre-pairing). The offspring LOAEL is 600 ppm or 48.0 mg/kg/day for males and 250 ppm or 22.0 mg/kg/day for females. In males the liver weight adjusted for body weight was increased with centrilobular hepatocellular hypertrophy in both generations. There was also an increased incidence of cell hypertrophy in the pars distalis of the pituitary in the F1 generation. In females liver weight adjusted for body weight was increased in the F1 generation only. In the pups at 250 ppm, body weight was reduced in both generations. In F1 male pups and F1 and F2 female pups, the weight of the liver adjusted for body weight was statistically significantly increased. In F1 male pups, the weight of the spleen adjusted for the body weight was statistically significantly reduced. A higher number of

dams were in “lactational diestrus” at day 21 of lactation. The time until preputial separation in F1 male pups was increased.

The NOAEL for reproduction was 600 ppm in males or 40.5 mg/kg/day for P generation males during pre-pairing and 250 ppm or 19.4 mg/kg/day in females. The LOAEL for reproduction in both genders was not determined, since the NOAEL was the HDT.

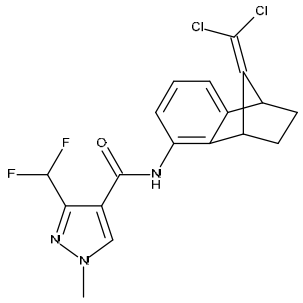
This study is **acceptable/non-guideline** and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800; OECD 416) in rats. However, critical parameters (ovarian follicle counts) were not assessed at 25 or 100 ppm and consequently, a reproductive NOAEL could not be established in females.

Compliance: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No claim of CBI was made for any information contained in this document.

I. MATERIALS AND METHODS

A. Materials:

Test Material: SYN545192



Description:	Beige powder
Lot/Batch number:	SMU9BP005
Purity:	97%
Stability of test compound:	Expiry date: 28-Feb-2013
Vehicle:	None. The test substance was administered via the diet.
Positive control:	None

Test Animals:

Species	Rat
Strain	HanRcc: WIST(SPF)
Age at dosing	Males: 7 weeks Females: 7 weeks
Weight at dosing	Males: 205 to 257 g Females: 136 to 176 g
Source	Harlan Laboratories, B.V. Kreuzelweg 53. 5961 NM Horst, Netherlands
Housing	Individually in Makrolon type-3 cages (except during mating) with wire mesh tops and sterilized standard softwood bedding
Acclimatisation period	5 days
Diet	Granulated standard Kliba-Nafag 3433 rat/mouse maintenance diet (Provimi Kliba AG, 4303 Kaiseraugst/Switzerland) was available *ad libitum*
Water	Community tap-water from Füllinsdorf was available *ad libitum* in water bottles.
Environmental conditions	Temperature: 22 ±3°C Humidity: 30-70% Air changes: Air-conditioned with 10-15 air changes per hour Photoperiod: 12-hour fluorescent light / 12-hour dark cycle Other: music during the light period. A wood stick was provided for environmental enrichment.

B. Study Design and Methods:

1. In-life dates: Start: 18-May-2010 End: 04-Feb-2011

2. Mating procedure: Vaginal smears were taken for 21 days immediately prior to pairing and throughout the pairing period until evidence of mating was detected. After the pre-pairing period, females were housed with sexually mature males (1:1) until evidence of copulation was observed. Siblings were not paired. The females were removed and housed individually if the daily vaginal smear was sperm positive, or a copulation plug was observed. The day of mating was designated day 0 post coitum. A period of 14 days was allowed for mating. Any female for which no evidence of mating was detected after 14 days was housed individually.

3. Study schedule: For the P generation, the test item was administered over a 70-day pre-pairing period and during the pairing and after pairing periods in males and during the pairing, gestation and lactation periods in females for breeding of the F1 litters. All P dams were allowed to give birth and rear their litters (F1 pups) up to day 21 post partum (weaning), after which the dams were separated from their pups. Day 0 was designated as the day on which a female had delivered all her pups. The offspring were examined as soon as possible after completion of delivery for litter size, number of live and still births, and any gross abnormalities.

Following weaning of the F1 litters on day 21 post partum, F1 animals were selected for the next generation. Treatment was considered to have commenced when the selected F1

animals were about four weeks of age but the animals were maintained on their respective diets from weaning. The test item was administered during growth of the F1 generation to adulthood (at least a 70-day pre-pairing period) and also during the pairing, gestation and lactation periods for breeding of the F2 litters. After weaning, the F1 generation of 25 male and 25 female pups per dose level was randomly selected from as many different litters as possible, retained for at least 70 days, then paired in automatic mating cages as above. Siblings were not paired.

All F1 dams were allowed to give birth and rear their litters (F2 pups) up to day 21 post partum (weaning), after which the dams were separated from their pups. Day 0 was designated as the day on which a female had delivered all her pups. The offspring were examined as soon as possible after completion of delivery for litter size, number of live and still births, and any gross abnormalities. In case of a treatment-related effect in F1 sex ratio or sexual maturation, anogenital distance was measured on post natal day 1 in F2 pups.

Days	**Periods**		**Generations**
0	Treatment of P begins		
	:	P	
	:	:	
	: Pre-pairing	:	
	:	:	
	:	:	
70	- :		
	: Pairing	:	
	-	:	
	: Gestation	:	
	-	:	F1
	: Lactation	:	:
126 (approx.)	- Weaning Treatment of P ends and selected F1 begins	: :	: :
	:		:

	:	:	
	: Pre-pairing	:	
	:	:	
	:	:	
217 - (approx.)	:	:	
	: Pairing	:	
	-	:	
	: Gestation	:	
	-	:	F2
273 (approx.)	: Lactation	: :	:
294 (approx.)	: Weaning - Treatment of F1 ends	:	:

4. <u>Animal assignment</u>: Parental animals were assigned to test groups using a computer-generated random algorithm. In addition body weights (recorded on the day of allocation) were taken into consideration in order to ensure similar mean body weights in all groups. Selection of F1 and F2 pups for different allocations (culling on day 4 post partum, necropsy and breeding) were based on randomly allocated pup numbers on day 1 post partum.

Table IIA 5.6.1/02–1: Animal Assignment[a]

Test item intake of males - pre-pairing period

Generation	Concentration (ppm)	Mean achieved dose level (mg/kg bw/day)
P	0	0
	25	1.7
	100	6.8
	600	40.5

Test item intake of males - post-pairing period

Generation	Concentration (ppm)	Mean achieved dose level (mg/kg bw/day)
P	0	0
	25	1.2
	100	4.9
	600	29.7

Test item intake of females - pre-pairing period

Generation	Concentration (ppm)	Mean achieved dose level (mg/kg bw/day)
P	0	0
	25	2.0
	100	8.2
	250	19.4

Test item intake of females - gestation period

Generation	Concentration (ppm)	Mean achieved dose level (mg/kg bw/day)
P	0	0
	25	1.9
	100	7.6
	250	17.5

Test item intake of females - lactation period

Generation	Concentration (ppm)	Mean achieved dose level (mg/kg bw/day)
P	0	0
	25	4.1
	100	16.8
	250	40.2

a Data obtained from summaries on pages 45-46 in the study report.

5. <u>Dose selection rationale</u>: Dietary concentrations for this study were based on the results of a preliminary single generation study in Han Wistar rats, Harlan Laboratories Study C56928, which used dietary levels of 0, 75, 200 and 400 ppm for the females and 0, 75, 400 and 600 ppm for the males with the same batch no. of SYN545192. A dietary concentration level of 400 ppm in females was demonstrated to be too high for use in the two generation study due to significantly reduced body weight in dams and offspring. There was no significant effect on body weight at 200 ppm in females, although food consumption was occasionally reduced. All dietary concentrations were well tolerated in males. Dietary concentration levels of 0, 25, 100 and 250 ppm for females and 0, 25, 100 and 600 ppm for males were used for this study.

6. <u>Diet/dosage preparation and analysis</u>: Dietary admixtures were prepared every third week using the test item as supplied by the Sponsor. The test item was mixed to

granulated diet. The test item was weighed into a tared glass beaker on a suitable precision balance, and mixed with micro-granulated feed separately for each dose group. Control feed for the animals of group 1 was prepared similarly, but without test item. The results indicate the accurate use of the test item SYN545192 and the control diet during this study.

Results -

Homogeneity Analysis: Analysis confirmed the homogeneity of the dose preparations which did not deviate by more than 7.8% (<10%) from the corresponding mean.

Concentration analysis: The individual diet samples analyzed during the study were found to contain SYN545192 in the range of 90.2% to 109.8% of nominal and, thus, the required content limit of ±10% of the nominal concentration was met.

Stability analysis: the test item was found to be stable in diet when kept for 28 days at room temperature based on recoveries that were within 10% of the time-zero (homogeneity) mean.

C. Methods

1. Parental animals: Observations and the schedule for those observations are summarized from the report as follows:

Observation	Frequency
Mortality and morbidity:	Twice daily
Clinical signs:	Daily cage-side clinical observations. Detailed clinical observations were performed on a weekly basis outside the home cage at the same time as the measurement of body weight. Animals were observed for the following: changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions, and autonomic activity (e.g. lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypes or bizarre behavior were also reported.
Body weight:	All animals were weighed on the first day of dosing and thereafter at weekly intervals, with the exception of the pairing period. After mating, females were weighed on days 0, 7, 14 and 21 post coitum. Dams which littered were weighed on days 1, 4, 7, 14 and 21 post partum and on the day the animals were sacrificed.
Food consumption and compound intake:	Food consumption was recorded for 3 periods per week at intervals of 2 or 3 days during the pre-pairing, gestation and lactation periods up to day 21 post partum. Food utilization and intake of the test item expressed as mg/kg/day were calculated.
Determination of the estrous cycle stage:	For P and F1 generation, 21 days prior to pairing and throughout pairing until the smear was sperm-positive or a copulation plug was observed. A vaginal smear was taken immediately before termination of each female.
Sperm analysis:	**Motility** At necropsy of adult males an epididymal sperm sample was obtained from the left cauda epididymidis of each male. The sample was diluted with a pre-warmed (about 35 °C) physiological medium, and shortly after being obtained, one hundred sperm were counted microscopically for determination of percentage of not motile, stationary motile and progressively motile sperm. **Morphology** A sperm sample from the left vas deferens was used for morphological

	assessment after fixation and Eosin staining. 500 sperm per sample were evaluated microscopically and classified into the following categories: Code Description A Normal, complete sperm B Normal head only (tail detached) C Complete sperm, misshapen hook D Complete sperm, abnormally curved hook E Complete sperm, reversed head F Abnormal head only (tail detached) Morphological sperm evaluation was performed initially only for group 1 and 4 males. In the absence of a treatment-related effect the slides for the group 2 and 3 males were not evaluated. **Sperm, spermatid count** The left caudal epididymis and left testis were taken for determination of homogenization resistant spermatids and caudal epididymal sperm reserve. These tissues were frozen at -20 ± 5 °C pending evaluation. For evaluation the weighed tissues were placed in Triton-X-100 solution and homogenized with a blender (Ultra Turrax) and an ultrasonic water bath. Sperm or spermatid heads were counted microscopically using a modified Neubauer chamber. These evaluations were performed in the first instance only for group 1 and 4 males. In the absence of a treatment-related effect the remaining frozen tissues were not evaluated.

On day 4 postpartum, litters were standardized to a maximum of 8 pups per litter (4/sex/litter, as nearly as possible); excess pups were euthanized and discarded. Culling was not conducted on litters of less than 8 pups.

3. Postmortem observations

a) Parental animals:

All animals were sacrificed by an injection of sodium pentobarbital and examined macroscopically for any structural abnormalities or pathological changes. Vaginal smears were taken from all P and F1 dams. All animals sacrificed or found dead were subjected to a detailed macroscopic examination to establish, if possible, the cause of death. Specimens of abnormal tissue were fixed in neutral phosphate buffered 4% formaldehyde solution. Dead pups, except those excessively cannibalized, were examined macroscopically. All dead pups were fixed in neutral phosphate buffered 4% formaldehyde solution. For the parent animals, special attention was directed at the organs of the reproductive system. The number of corpora lutea and implantation sites was noted for all mated females. The uteri of all dams, in which implantation sites were not visible, were placed in a solution of ammonium sulfide to visualize possible hemorrhagic areas of implantation sites and the number of implantation sites were noted.

The following organ weights were recorded for all P and F1 parental males and females on day 21 post partum, or shortly thereafter:

Brain (incl. entire brainstem)	Kidney
Pituitary	Adrenal glands

Liver	Spleen
Thyroid	Testes
Seminal vesicles with coagulating glands and their fluids (as one unit)	Epididymides (total weight as cauda separately)
Prostate	Ovaries
Uterus (including cervix, excluding oviducts)	

Paired organs were weighed separately.

The following tissues from all P and F1 parental males and females were collected at necropsy and fixed in neutral phosphate buffered 4% formaldehyde solution:

Gross lesions	Pituitary
Adrenal glands	Liver
Prostate	
Seminal vesicles with coagulating gland	Right testis and epididymidis (in Bouin's fixative)
Ovaries	Uterus and cervix
Vagina	Oviducts

Adult animals - all organs not listed under tissue preservation, but which were to be weighed at necropsy from the selected adult animals, were fixed in neutral phosphate buffered 4% formaldehyde solution in case a subsequent weight difference was identified. One male and one female pup per litter selected for organ weights - abnormal tissues, organs weighed at necropsy and thyroids were fixed in neutral phosphate buffered 4% formaldehyde solution. Abnormal tissues were also preserved from the remaining male and female pups per litter, which were given a macroscopic examination.

All organ and tissue samples were processed, embedded and cut at an approximate thickness of 2 - 4 micrometers and stained with hematoxylin and eosin. For the respective testis, PAS staining was used. Special stains were used at the discretion of the study pathologist. Slides of all organs and tissues collected at terminal sacrifice from the parental animals of the control and high-dose groups were examined by the study pathologist. The same applied to all occurring gross lesions and to all animals, which died spontaneously or had to be terminated in extremis. Special emphasis was made on the stages of spermatogenesis and histopathology of interstitial cell structure.

In addition to qualitative examination of ovarian histopathology, a quantitative evaluation of primordial follicles, growing follicles and antral follicles from 10 sections per ovary, and corpora lutea from 1 section per ovary in 10 females of the F1 generations in groups 1 and 4 were included. If test item-related morphologic changes were detected in organs of any high-dose animal, those same organs from the mid- and low-dose group were examined to establish a no-effect level, if possible. Histological examination of ovaries was carried out on any females that did not give birth. In addition, microscopic examination of the reproductive organs of all infertile males was made, if necessary.

b) Offspring: The F_1 offspring not selected as parental animals and all F_2 offspring were sacrificed at 21 days of age (after weaning). These animals were subjected to examination post-mortem (macroscopic and/or microscopic examination).

The following organ weights were recorded from one randomly selected male and female pup from each F_1 and F_2 litter:

X	Brain	X	Spleen
X	Liver	X	Thymus

D. Data Analysis

1. Statistical analyses: The following statistical methods were used to analyze food consumption, body weight and reproduction data:

□□Means and standard deviations of various data were calculated.
□□All statistical tests were two-sided.
□□Statistical significance between groups was evaluated by Analysis of Variance (ANOVA). In the case where variances were non-homogenous, appropriate transformations were applied (e.g. log, square root, cube root or double arcsine) to stabilize the variances before the ANOVA. Dunnett many-one t-test was then used to compare each group to control based on the error mean square in the ANOVA.

Fisher's exact-test was applied if the variables could be dichotomized without loss of information.
□□Organ weights were analyzed using ANOVA as above and by analysis of covariance (ANCOVA) using terminal kill body weight as covariate. Ratios of organ weight/body weight and organ weight/brain weight are presented for information, but were not analyzed statistically.

3. Historical control data – Historical control data for pregnancy, litter and implantation data, pup weights, sexual maturation and organ weights are presented on pages 705-772.

II. RESULTS

A. Parental animals:

1. Clinical signs of toxicity: No significant test item-related clinical signs were noted in the males or females at any dose level. Findings observed included hair loss, scabs and wounds, and a kinked tail. No test item-related findings were observed. In the males, findings included hair loss, wounds, crusts, a kinked tail and flecks in the eye. One male in the 600 ppm group produced vocalizations in weeks 7 - 10 of the pre-pairing period. In the females, findings included hair loss, a kinked tail, wounds and crusts. One female in

the 100 ppm group had slight opacity in the left eye during the pre-pairing period. One female in the 25 ppm dose group had a reddened eye during the gestation period.

2. Mortality: One female in the 25 ppm group was killed for ethical reasons on day 9 post coitum. This female had scabs and wounds, which had spread across the face, neck and shoulders. One female in the 250 ppm group had ruffled fur on days 1 - 4 of the lactation period and was found dead on day 4 post partum. These incidences were isolated and considered not to be related to treatment with the test item.

3. Body weight and body weight gains:

At 600 ppm in males during the pre-pairing period, mean body weight was statistically significantly lower on days 22, 36 and 43 of the pre-pairing period. This was a reflection of the slightly lower body weight gain at this dose level during the pre-pairing period and was considered to be test item-related. In the 25 and 100 ppm groups, mean body weight was not affected by treatment with the test item with statistical significance.

At 600 ppm in males during the post-pairing period, mean body weight was lower than the control group during the whole of the post-pairing period and was statistically significantly reduced on days 8 and 15. These differences were a reflection of the lower body weight observed at this dose level at the end of the pre-pairing phase. At 25 and 100 ppm, mean body weight was not affected by treatment with the test article with statistical significance after the pairing period.

In females during the pre-pairing period, the mean body weight in the 250 ppm dose group was statistically significantly lower than controls from day 57 onwards. This reduction was a reflection of the reduced weight gain in this group consistent with the observed reduction in food consumption at this dose level and was considered to be test article-related. At 100 ppm, mean body weight was statistically significantly reduced on day 64 reflecting slightly lower weight gain in this group. This was considered to be a slight effect of the test item. At 25 ppm, mean body weight was not affected by treatment with the test article with statistical significance.

In the 250 ppm group females during the gestation period, mean body weight was statistically significantly lower than control throughout the gestation period. In the 100 ppm group, mean body weight was lower than control throughout the gestation period and was statistically significantly reduced over days 0 - 14 post coitum. In the 25 ppm group, mean body weight was not affected by treatment with the test article with statistical significance.

In the females in the 250 ppm group during lactation, mean body weight was statistically significantly lower than control throughout the lactation period. In the 100 ppm group, mean body weight was lower than control throughout the lactation period and was statistically significantly reduced over days 1 - 7 post partum. In the 25 ppm group, mean body weight was not affected by treatment with the test article with statistical significance.

The female parental body weight was decreased by 0.5-7.8% (mean= -4.0%) during the 70-day pre-pairing period at the top dose, not considered adverse nor large nor excessive weight loss. The female parental body weight was decreased by 9.7-13.1% (mean= -11.0%) during the gestation period at the top dose, considered adverse but not large nor excessive. The female parental body weight during lactation was decreased by 9.6-14.7% (mean= -11.8%) at the top dose, considered adverse but not large nor excessive. The time-based composite decrease in body weight for the parental females during the entire study was a 6.8% reduction, not considered to be large nor excessive.

BODY WEIGHTS (G)
MALES

		Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 600 ppm
PRE- PAIRING PERIOD					
Day 1	MEAN	226	223 -	220 -	222 -
	ST.DEV.	13	9	12	11
	N	25	25	25	25
Day 8	MEAN	266	263 -	259 -	257 -
	ST.DEV.	16	13	15	14
	N	25	25	25	25
Day 15	MEAN	300	296 -	294 -	290 -
	ST.DEV.	17	17	21	15
	N	25	25	25	25
Day 22	MEAN	327	321 -	319 -	313 *
	ST.DEV.	18	20	23	17
	N	25	25	25	25
Day 29	MEAN	348	340 -	339 -	334 -
	ST.DEV.	18	23	26	20
	N	25	25	25	25
Day 36	MEAN	367	357 -	357 -	350 *
	ST.DEV.	21	25	29	23
	N	25	25	25	25
Day 43	MEAN	380	372 -	371 -	362 *
	ST.DEV.	22	28	33	23
	N	25	25	25	25
Day 50	MEAN	396	386 -	387 -	378 -
	ST.DEV.	23	29	34	26
	N	25	25	25	25
Day 57	MEAN	408	396 -	399 -	388 -
	ST.DEV.	23	31	35	27
	N	25	25	25	25
Day 64	MEAN	418	405 -	409 -	398 -
	ST.DEV.	24	33	37	28
	N	25	25	25	25
Day 70	MEAN	428	413 -	417 -	406 -
	ST.DEV.	25	35	37	29
	N	25	25	25	25

BODY WEIGHTS (GRAM) OF MALES

P GENERATION - AFTER PAIRING PERIOD

			GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 600 PPM
DAY	1	MEAN	440	427	432	419
		ST.DEV.	23.7	36.4	37.2	32.6
		N	25	25	25	25
DAY	8	MEAN	450	435	438	427 *
		ST.DEV.	24.3	37.0	38.0	33.9
		N	25	25	25	25
DAY	15	MEAN	456	442	445	432 *
		ST.DEV.	25.1	37.2	39.2	34.2
		N	25	25	25	25
DAY	22	MEAN	464	448	451	440
		ST.DEV.	27.3	38.1	41.5	35.0
		N	25	25	25	25
DAY	29	MEAN	472	456	460	448
		ST.DEV.	27.5	38.6	42.3	35.9
		N	25	25	25	25
DAY	36	MEAN	476	461	465	453
		ST.DEV.	28.2	39.4	43.1	37.1
		N	25	25	25	25
DAY	43	MEAN	480	462	469	459
		ST.DEV.	28.0	40.8	45.3	39.1
		N	25	25	25	25
DAY	44	MEAN	479	484	460	448
		ST.DEV.	29.4	47.8	27.6	26.8
		N	8	9	8	8
DAY	45	MEAN	487	456	495	463
		ST.DEV.	34.3	40.6	44.1	53.1
		N	9	8	8	9

BODY WEIGHTS (G)
FEMALES

		Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 250 ppm
PRE- PAIRING PERIOD					
Day 1	MEAN	159	160 -	158 -	160 -
	ST.DEV.	9	7	5	7
	N	25	25	25	25
Day 8	MEAN	179	180 -	175 -	177 -
	ST.DEV.	8	9	7	8
	N	25	25	25	25
Day 15	MEAN	192	194 -	190 -	191 -
	ST.DEV.	10	11	8	9
	N	25	25	25	25
Day 22	MEAN	202	206 -	201 -	199 -
	ST.DEV.	11	13	11	10
	N	25	25	25	25
Day 29	MEAN	214	216 -	211 -	207 -
	ST.DEV.	13	14	11	11
	N	25	25	25	25
Day 36	MEAN	220	224 -	218 -	215 -
	ST.DEV.	15	15	12	12
	N	25	25	25	25
Day 43	MEAN	224	227 -	222 -	218 -
	ST.DEV.	16	16	13	13
	N	25	25	25	25
Day 50	MEAN	231	236 -	228 -	223 -
	ST.DEV.	16	18	14	13
	N	25	25	25	25
Day 57	MEAN	249	248 -	238 -	231 **
	ST.DEV.	17	19	13	15
	N	25	25	25	25
Day 64	MEAN	255	249 -	241 *	235 **
	ST.DEV.	19	22	13	16
	N	25	25	25	25
Day 70	MEAN	255	251 -	244 -	235 **
	ST.DEV.	20	22	13	15
	N	25	25	25	25

BODY WEIGHTS (GRAM) OF FEMALES
P GENERATION - GESTATION PERIOD

			GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
DAY	0	MEAN	257	250	243 *	232 **
		ST.DEV.	18.3	22.6	14.2	15.2
		N	23	20	23	22
DAY	7	MEAN	279	272	265 *	252 **
		ST.DEV.	18.8	23.6	12.1	16.2
		N	23	20	23	22
DAY	14	MEAN	306	297	291 *	271 **
		ST.DEV.	21.0	25.8	13.4	17.9
		N	23	20	23	22
DAY	21	MEAN	374	366	356	325 **
		ST.DEV.	30.8	33.4	23.4	28.1
		N	23	20	23	22

BODY WEIGHTS (GRAM) OF FEMALES
P GENERATION - LACTATION PERIOD

			GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
DAY	1	MEAN	286	279	263 **	244 **
		ST.DEV.	18.3	23.8	13.1	18.4
		N	23	20	23	22
DAY	4	MEAN	295	290	279 *	259 **
		ST.DEV.	18.4	26.6	18.0	20.2
		N	23	20	23	22
DAY	7	MEAN	304	299	287 *	269 **
		ST.DEV.	18.9	26.1	17.5	21.4
		N	23	20	23	22
DAY	14	MEAN	302	303	297	273 **
		ST.DEV.	12.6	18.9	14.4	23.0
		N	23	20	23	22
DAY	21	MEAN	299	293	287	266 **
		ST.DEV.	14.2	20.8	11.9	25.3
		N	23	20	23	22

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

4. <u>Food consumption</u>: In males in the 600 ppm group during the pre-pairing period, mean food consumption was statistically significantly reduced over days 1 - 3 and 68 - 70 of the pre-pairing period. This slight effect was considered to be related to treatment with the test item. In the 25 and 100 ppm groups, mean food consumption was not affected by treatment with the test article with statistical significance.

In the males in the 600 ppm group after pairing, mean food consumption was statistically significantly reduced over days 1 - 3. For the remainder of the after pairing period, mean food consumption was similar to the control group. In the 25 and 100 ppm groups, mean food consumption was not affected by treatment with the test article with statistical significance.

In the females in the 250 ppm dose group during the pre-pairing period, mean food consumption was lower than the control group throughout most of the pre-pairing period. Food consumption was statistically significantly lower than control over days 1 - 5, 36 -

38, 40 - 43, 47 - 50 as well as days 52 - 66 of the pre-pairing period. This reduction was considered to be an effect of treatment with the test item. In the 25 and 100 ppm dose groups, mean food consumption was not affected by treatment with the test item with statistical significance.

In the females in the 250 ppm dose group during gestation, mean food consumption was statistically significantly lower than control throughout the gestation period. This was considered to be a test item-related effect. In the 25 and 100 ppm dose groups, mean food consumption was not affected by treatment with the test article with statistical significance.

In the females in the 250 ppm group during lactation, mean food consumption was lower than the control group throughout the lactation period and was statistically significantly reduced over the period days 7 - 19 post partum. In the 25 and 100 ppm dose groups, mean food consumption was not affected by treatment with the test item during the lactation period with statistical significance.

In the parental females, the reduced food consumption of 0-21% during the pre-pairing, gestation and lactation periods at the high dose were similar magnitudes as the body weight decreases and may explain the decreased body weights.

FOOD CONSUMPTION (G/ANIMAL/DAY)
MALES

		Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 600 ppm
PRE- PAIRING PERIOD					
Days 1-3	MEAN	20.9	20.7 -	20.9 -	18.0 **
	ST.DEV.	2.3	1.4	1.6	1.5
	N	25	25	25	25
Days 3-5	MEAN	22.2	22.3 -	22.4 -	21.3 -
	ST.DEV.	2.0	2.0	1.8	2.0
	N	25	25	25	25
Days 5-8	MEAN	22.8	23.7 -	23.4 -	22.3 -
	ST.DEV.	2.3	2.1	2.0	1.9
	N	25	25	25	25
Days 8-10	MEAN	22.3	22.3 -	22.4 -	21.6 -
	ST.DEV.	1.8	1.7	1.9	1.9
	N	25	25	25	25
Days 10-12	MEAN	23.5	23.3 -	23.3 -	22.4 -
	ST.DEV.	2.3	1.9	2.4	1.9
	N	25	25	25	25
Days 12-15	MEAN	23.1	23.0 -	23.0 -	22.0 -
	ST.DEV.	2.1	1.6	2.1	1.6
	N	25	25	25	25
Days 15-17	MEAN	23.0	22.7 -	22.5 -	22.0 -
	ST.DEV.	1.8	1.7	1.8	1.4
	N	25	25	25	25
Days 17-19	MEAN	23.6	23.1 -	23.2 -	22.5 -
	ST.DEV.	1.9	1.9	2.3	1.8
	N	25	25	25	25
Days 19-22	MEAN	23.4	22.8 -	22.8 -	22.6 -
	ST.DEV.	1.8	2.0	2.0	1.8
	N	25	25	25	25
Days 22-24	MEAN	21.7	21.6 -	21.7 -	21.6 -
	ST.DEV.	1.4	2.2	2.0	1.9
	N	25	25	25	25
Days 24-26	MEAN	23.3	22.6 -	22.7 -	22.4 -
	ST.DEV.	1.7	1.9	2.1	2.1
	N	25	25	25	25

		Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 600 ppm
PRE- PAIRING PERIOD					
Days 26-29	MEAN	23.3	22.8 -	23.3 -	22.8 -
	ST.DEV.	1.6	1.9	2.4	1.9
	N	25	25	25	25
Days 29-31	MEAN	22.4	23.2 -	23.2 -	22.4 -
	ST.DEV.	1.2	2.4	2.3	2.0
	N	25	25	25	25
Days 31-33	MEAN	23.5	23.1 -	23.3 -	22.8 -
	ST.DEV.	1.9	2.1	2.3	1.9
	N	25	25	25	25
Days 33-36	MEAN	23.3	22.8 -	23.1 -	22.2 -
	ST.DEV.	1.9	1.8	2.4	1.9
	N	25	25	25	25
Days 36-38	MEAN	23.0	22.6 -	22.5 -	21.8 -
	ST.DEV.	1.9	2.1	2.3	1.9
	N	25	25	25	25
Days 38-40	MEAN	22.9	22.4 -	22.6 -	22.0 -
	ST.DEV.	1.6	2.3	2.3	2.1
	N	25	25	25	25
Days 40-43	MEAN	21.8	21.9 -	22.2 -	21.3 -
	ST.DEV.	2.1	1.8	2.4	2.8
	N	25	25	25	25
Days 43-45	MEAN	23.2	23.5 -	23.3 -	23.3 -
	ST.DEV.	1.5	1.8	2.5	3.1
	N	25	25	25	25
Days 45-47	MEAN	23.0	22.7 -	22.7 -	23.0 -
	ST.DEV.	1.9	2.1	2.3	2.3
	N	25	25	25	25
Days 47-50	MEAN	23.4	22.4 -	22.8 -	22.8 -
	ST.DEV.	2.0	1.8	2.2	1.9
	N	25	25	25	25
Days 50-52	MEAN	22.2	22.3 -	22.7 -	22.4 -
	ST.DEV.	1.9	2.3	2.2	2.0
	N	25	25	25	25

		Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 600 ppm
PRE- PAIRING PERIOD					
Days 52-54	MEAN	23.4	22.7 -	23.3 -	22.7 -
	ST.DEV.	1.8	2.1	2.1	2.4
	N	25	25	25	25
Days 54-57	MEAN	23.2	22.5 -	22.8 -	22.7 -
	ST.DEV.	1.7	1.9	2.1	2.0
	N	25	25	25	25
Days 57-59	MEAN	21.5	21.9 -	22.9 *	21.9 -
	ST.DEV.	1.6	1.8	2.0	2.1
	N	25	25	25	25
Days 59-61	MEAN	23.6	23.0 -	23.2 -	22.5 -
	ST.DEV.	2.3	2.4	2.4	1.9
	N	25	25	25	25
Days 61-64	MEAN	22.5	22.4 -	22.3 -	21.8 -
	ST.DEV.	1.7	1.8	1.7	1.9
	N	25	25	25	25
Days 64-66	MEAN	21.2	21.5 -	21.5 -	21.0 -
	ST.DEV.	1.3	2.1	1.8	2.1
	N	25	25	25	25
Days 66-68	MEAN	22.4	21.7 -	21.9 -	21.7 -
	ST.DEV.	1.9	2.0	2.2	1.8
	N	25	25	25	25
Days 68-70	MEAN	23.7	22.5 -	23.0 -	22.1 *
	ST.DEV.	2.0	2.1	1.8	1.8
	N	25	25	25	25

*/**/- : Dunnett-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

FOOD CONSUMPTION OF MALES
(G/ANIMAL/DAY)
P GENERATION - AFTER PAIRING PERIOD

			GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 600 PPM
DAYS	1-3	MEAN	22.9	22.5	21.8	21.4 *
		ST.DEV.	1.7	2.0	1.8	2.7
		N	25	25	25	25
DAYS	3-5	MEAN	22.3	22.1	21.6	21.5
		ST.DEV.	1.7	2.1	2.2	2.1
		N	25	25	25	25
DAYS	5-8	MEAN	21.9	21.8	21.2	21.6
		ST.DEV.	1.4	2.0	1.9	1.9
		N	25	25	25	25
DAYS	8-10	MEAN	21.6	22.0	21.6	21.4
		ST.DEV.	1.8	2.0	2.1	2.0
		N	25	25	25	25
DAYS	10-12	MEAN	23.5	23.0	23.1	22.6
		ST.DEV.	1.9	2.0	2.4	2.1
		N	25	25	25	25
DAYS	12-15	MEAN	22.7	22.3	22.5	22.5
		ST.DEV.	1.8	1.6	2.2	2.0
		N	25	25	25	25
DAYS	15-17	MEAN	21.7	21.8	21.3	21.2
		ST.DEV.	1.8	1.9	2.1	2.2
		N	25	25	25	25
DAYS	17-19	MEAN	23.1	23.0	23.5	22.7
		ST.DEV.	2.2	2.2	2.8	2.2
		N	25	25	25	25
DAYS	19-22	MEAN	22.1	21.7	22.1	21.8
		ST.DEV.	2.1	1.8	2.8	2.2
		N	25	25	25	25
DAYS	22-24	MEAN	21.5	20.9	21.4	21.2
		ST.DEV.	2.0	1.9	2.6	2.2
		N	25	25	25	25
DAYS	24-26	MEAN	21.0	21.1	22.0	20.8
		ST.DEV.	1.7	2.3	2.7	2.4
		N	25	25	25	25
DAYS	26-29	MEAN	21.9	21.2	21.7	21.2
		ST.DEV.	1.7	1.7	2.3	2.0
		N	25	25	25	25
DAYS	29-31	MEAN	22.6	22.3	22.1	21.9
		ST.DEV.	2.1	1.6	1.9	2.3
		N	25	25	25	25
DAYS	31-33	MEAN	22.0	21.5	21.5	21.6
		ST.DEV.	2.1	2.0	2.4	2.1
		N	25	25	25	25
DAYS	33-36	MEAN	21.5	21.2	21.5	21.8
		ST.DEV.	1.8	1.7	2.0	2.0
		N	25	25	25	25

* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 600 PPM
DAYS 36-38	MEAN	21.3	21.2	21.4	21.2
	ST.DEV.	1.7	1.9	2.1	2.3
	N	25	25	25	25
DAYS 38-40	MEAN	23.1	22.2	23.1	23.1
	ST.DEV.	1.9	1.6	2.8	2.6
	N	25	25	25	25
DAYS 40-43	MEAN	20.3	19.5	20.3	20.2
	ST.DEV.	1.6	1.9	2.3	2.0
	N	25	25	25	25

FOOD CONSUMPTION (G/ANIMAL/DAY)
FEMALES

		Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 250 ppm
PRE- PAIRING PERIOD					
Days 1-3	MEAN	14.9	15.4 -	14.8 -	12.8 **
	ST.DEV.	1.5	1.4	1.5	1.2
	N	25	25	25	25
Days 3-5	MEAN	16.5	16.6 -	16.1 -	15.5 *
	ST.DEV.	1.7	1.6	1.2	1.2
	N	25	25	25	25
Days 5-8	MEAN	16.8	17.3 -	16.9 -	16.2 -
	ST.DEV.	1.7	1.5	1.2	1.2
	N	25	25	25	25
Days 8-10	MEAN	16.3	16.5 -	16.5 -	15.9 -
	ST.DEV.	1.9	2.0	1.5	1.1
	N	25	25	25	25
Days 10-12	MEAN	17.3	17.4 -	17.0 -	16.1 *
	ST.DEV.	1.7	1.7	1.4	1.2
	N	25	25	25	25
Days 12-15	MEAN	17.0	17.3 -	17.2 -	16.2 -
	ST.DEV.	1.8	1.7	1.2	1.2
	N	25	25	25	25
Days 15-17	MEAN	17.3	17.0 -	16.6 -	16.2 -
	ST.DEV.	1.8	2.3	1.4	1.4
	N	25	25	25	25
Days 17-19	MEAN	17.0	17.4 -	17.4 -	16.3 -
	ST.DEV.	1.9	1.8	1.7	1.4
	N	25	25	25	25
Days 19-22	MEAN	17.2	17.2 -	17.0 -	16.2 -
	ST.DEV.	1.9	1.7	1.4	1.5
	N	25	25	25	25
Days 22-24	MEAN	15.6	15.9 -	16.3 -	15.7 -
	ST.DEV.	1.7	1.5	1.6	1.7
	N	25	25	25	25
Days 24-26	MEAN	16.7	17.0 -	18.2 *	16.7 -
	ST.DEV.	1.7	1.9	1.9	3.0
	N	25	25	25	25

		Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 250 ppm
PRE- PAIRING PERIOD					
Days 26-29	MEAN	17.5	17.7 -	18.1 -	16.6 -
	ST.DEV.	1.8	1.8	1.4	1.2
	N	25	25	25	25
Days 29-31	MEAN	16.5	17.7 -	17.7 -	16.9 -
	ST.DEV.	1.8	2.2	1.7	1.9
	N	25	25	25	25
Days 31-33	MEAN	18.7	18.1 -	17.7 -	16.7 **
	ST.DEV.	2.1	2.0	1.5	1.6
	N	25	25	25	25
Days 33-36	MEAN	17.1	17.1 -	17.3 -	16.2 -
	ST.DEV.	1.6	1.7	1.2	1.1
	N	25	25	25	25
Days 36-38	MEAN	17.0	17.4 -	16.7 -	15.4 *
	ST.DEV.	2.1	2.3	1.5	1.6
	N	25	25	25	25
Days 38-40	MEAN	17.1	17.1 -	17.1 -	16.1 -
	ST.DEV.	2.1	1.7	1.6	1.6
	N	25	25	25	25
Days 40-43	MEAN	17.0	16.8 -	16.9 -	15.8 *
	ST.DEV.	1.8	1.7	1.3	1.2
	N	25	25	25	25
Days 43-45	MEAN	17.1	17.6 -	17.2 -	16.0 -
	ST.DEV.	1.8	2.2	1.7	1.8
	N	25	25	25	25
Days 45-47	MEAN	15.6	16.5 -	16.9 *	15.4 -
	ST.DEV.	1.7	1.5	1.6	1.2
	N	25	25	25	25
Days 47-50	MEAN	17.5	17.8 -	17.3 -	15.9 **
	ST.DEV.	1.9	2.0	1.4	1.4
	N	25	25	25	25
Days 50-52	MEAN	16.4	16.4 -	16.6 -	15.6 -
	ST.DEV.	1.9	2.0	1.3	1.9
	N	25	25	25	25

		Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 250 ppm
PRE- PAIRING PERIOD					
Days 52-54	MEAN	18.4	18.3 -	17.3 -	15.8 **
	ST.DEV.	2.2	2.2	1.8	1.7
	N	25	25	25	25
Days 54-57	MEAN	19.8	18.4 -	18.2 *	16.5 **
	ST.DEV.	2.6	2.4	1.5	1.6
	N	25	25	25	25
Days 57-59	MEAN	19.2	18.3 -	17.7 -	16.6 **
	ST.DEV.	2.7	3.0	2.4	2.3
	N	25	25	25	25
Days 59-61	MEAN	20.4	19.0 -	19.0 -	17.1 **
	ST.DEV.	3.1	2.9	2.6	2.6
	N	25	25	25	25
Days 61-64	MEAN	18.2	17.1 -	17.0 -	15.8 **
	ST.DEV.	3.1	2.7	2.5	1.5
	N	25	25	25	25
Days 64-66	MEAN	17.3	16.1 -	15.6 -	14.7 *
	ST.DEV.	3.8	2.8	3.5	1.7
	N	25	25	25	25
Days 66-68	MEAN	16.3	15.7 -	16.5 -	15.3 -
	ST.DEV.	2.7	2.0	1.7	1.4
	N	25	25	25	25
Days 68-70	MEAN	16.6	16.9 -	17.1 -	15.7 -
	ST.DEV.	2.5	2.4	1.7	1.6
	N	25	25	25	25

*/**/- : Dunnett-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

FOOD CONSUMPTION OF FEMALES
(G/ANIMAL/DAY)
P GENERATION - GESTATION PERIOD

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
DAYS 0-2	MEAN	17.7	17.6	17.3	15.6 **
	ST.DEV.	2.8	2.5	1.6	2.1
	N	23	20	23	22
DAYS 2-4	MEAN	20.9	20.1	19.4	17.9 **
	ST.DEV.	2.5	2.6	1.2	2.0
	N	23	20	23	22
DAYS 4-7	MEAN	21.2	20.7	20.5	18.4 **
	ST.DEV.	2.6	2.2	1.7	1.3
	N	23	20	23	22
DAYS 7-9	MEAN	22.2	21.6	21.3	18.2 **
	ST.DEV.	2.5	2.7	2.2	1.6
	N	23	20	23	22
DAYS 9-11	MEAN	22.6	22.0	21.9	18.7 **
	ST.DEV.	2.5	2.3	2.2	3.3
	N	23	20	23	22
DAYS 11-14	MEAN	23.0	22.8	22.8	19.6 **
	ST.DEV.	2.2	2.7	2.1	2.1
	N	23	20	23	22
DAYS 14-16	MEAN	22.5	22.0	21.6	18.6 **
	ST.DEV.	1.9	2.6	2.1	1.8
	N	23	20	23	22
DAYS 16-18	MEAN	24.0	23.1	22.8	19.1 **
	ST.DEV.	2.1	2.4	1.7	2.4
	N	23	20	23	22
DAYS 18-21	MEAN	22.9	23.1	22.0	18.1 **
	ST.DEV.	1.7	2.4	1.9	2.1
	N	23	20	23	22

Explanations for excluded data are listed in the tables of individual values
* / ** : Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level

FOOD CONSUMPTION (G/ANIMAL/DAY) OF FEMALES
P GENERATION - LACTATION PERIOD

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
DAYS 1-3	MEAN	25.0	26.2	27.2	24.8
	ST.DEV.	7.0	4.5	6.7	5.0
	N	23	20	23	22
DAYS 3-5	MEAN	37.9	38.4	38.8	35.8
	ST.DEV.	8.6	5.2	7.7	5.9
	N	23	20	23	22
DAYS 5-7	MEAN	41.1	42.3	42.1	40.9
	ST.DEV.	5.2	5.8	6.7	7.5
	N	23	20	23	22
DAYS 7-9	MEAN	47.0	47.0	47.3	41.3 **
	ST.DEV.	6.3	4.3	6.7	5.6
	N	23	20	23	22
DAYS 9-11	MEAN	52.7	51.3	50.8	42.7 **
	ST.DEV.	7.3	3.9	7.2	7.2
	N	23	20	23	22
DAYS 11-14	MEAN	51.8	52.0	52.4	45.6 **
	ST.DEV.	4.4	3.8	7.2	6.8
	N	23	20	23	22
DAYS 14-16	MEAN	58.4	56.1	58.0	50.4 **
	ST.DEV.	8.6	4.6	10.6	8.8
	N	23	20	23	22
DAYS 16-19	MEAN	54.0	53.2	54.1	47.0 *
	ST.DEV.	6.2	6.6	11.6	11.6
	N	23	20	23	22
DAYS 19-21	MEAN	62.6	64.7	64.1	56.8
	ST.DEV.	7.1	6.3	10.5	7.9
	N	23	20	23	21

5. Food Efficiency:

At 600 ppm in males during the pre-pairing period, mean food utilization was statistically significantly reduced over week 3 and week 5 of the pre-pairing period. At 25 ppm, mean food utilization was statistically significantly reduced in week 5. No other statistically significant changes were observed during the pre-pairing period at any dose level.

In females during the pre-pairing period, food utilization was statistically significantly lower in the 25, 100 and 250 ppm groups in week 8 and remained lower in week 9 in the 25 ppm group. The apparently lower values in treated groups in week 8 are likely to be due to the unexpectedly high value in the control group. No other statistically significant changes were observed during the pre-pairing period at any dose level.

6. Reproductive function:

a) Estrous cycle length and periodicity: The estrous cycle length was not affected by treatment with the test item. The number of irregular cycles (shortened) was statistically significantly lower in the 100 and 250 ppm groups compared to controls, however, the direction of the change this was not considered treatment-related. No dose-dependent change in mean days between estrous was observed.

b) Sperm measures: No test item-related effects were noted in motility. Statistical differences noted in the 100 ppm dose group were not observed in the high dose group and were considered unrelated to treatment. For morphology, The percentage of normal sperm at 600 ppm was statistically significantly lower than the control group (91.7% compared to 93.6% in the control group). However, this was within the range of the historical control data and was therefore considered to be incidental. The sperm head counts in the testis in the control group and in the 600 ppm group were similar and were within the range of the historical control data.

7. <u>Reproductive Performance</u>: Mating performance was not affected by treatment with the test article. Mean and median precoital times were similar in all dose groups. Fertility was also not affected by treatment with the test item. All females in all dose groups mated, with the exception of one female in the 25 ppm group. One female in the control group as well as three females in the 25 ppm group, two dams in the 100 ppm group and two dams in the 250 ppm group were not pregnant. One female in the control group had only implantations. One female in the 250 ppm group lost its whole litter and died subsequently.

MATING PERFORMANCE
P GENERATION

NUMBER OF FEMALES MATED DURING THE FIRST PAIRING PERIOD

Day of the pairing period	GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
1	3	3	7	7
2	8	8	2	1
3	3	6	8	6
4	6	4	5	8
5	1	1	-	-
6	-	1	-	-
7	2	1	1	2
10	-	-	1	-
12	2	-	-	-
14	-	-	1	-
Median precoital time	3	3	3	3
Mean precoital time	3.8	3.0	3.4	3.0
N	25	24	25	24
First mating not detected	-	-	-	1

FERTILITY
P GENERATION

FEMALES SCHEDULED FOR BREEDING

	GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
Percentage mating	100.0	96.0	100.0	100.0
Fertility index (%)	96.0	84.0	92.0	92.0
Conception rate (%)	96.0	87.5	92.0	92.0
Gestation index (%) (Breeding)	95.8	95.2	100.0	95.7

In the 250 ppm group, the mean corpora lutea count at necropsy was decreased by 14.4% with statistical significance in the P generation. At 100 ppm, the count was reduced by 5.4%, but without statistical significance. In the 25 ppm group, the mean corpora lutea count was similar to the control group.

CORPORA LUTEA COUNT
P GENERATION FEMALES

	GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
Corpora Lutea				
MEAN	16.7	16.0	15.8	14.3 **
ST.DEV.	3.3	2.5	2.4	1.7
N	23	20	23	22

The mean duration of gestation was similar in all dose groups (21.5 days in the control group compared to 21.6, 21.9 and 21.6 days in order of increasing dose level).

The mean number of implantations was slightly lower in the 250 ppm group (11.7 per dam compared to 13.2 in the control group). Although this was below the range of the historical control data, the difference to the controls was not statistically significant and there was not a clear dose related response. The mean number of implantations observed at 25 and 100 ppm (12.4 and 13.1 respectively) was comparable to controls. The total number of post-implantation losses was not affected by the test item (mean 1.7 in the control group compared to 1.3, 1.8 and 1.7 in order of ascending dose level).

The mean litter size at first litter check in the 250 ppm dose group was slightly lower than in the control group (10.0 compared to 11.5 in the control group), however, this was not statistically significant. In the 25 and 100 ppm dose groups, the mean litter size was similar to that of the control group. Mean litter size for the control group and all treated groups was below the historical control range of 11.7 to 12.9. Two dead pups were found at the first litter check in the control group, and 1 each at 25, 100 and 250 ppm and were considered to be incidental. No test item-related effect was observed in the postnatal loss. Five pups were lost in the control group compared to 1 each in the 25 and 100 ppm groups and 3 in the 250 ppm group. The losses were considered to be incidental due to the lack of dose-dependency. Breeding loss was not affected by treatment with the test item. One pup was lost in the control group as well as in the 100 and 250 ppm groups. There were no differences from control in birth index, viability index or weaning index at any dose level.

BREEDING DATA PER GROUP

P GENERATION

	GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
LITTERS				
TOTAL	23	20	23	23
EXCLUDED	0	0	0	1
EVALUATED	23	20	23	22
DURATION OF GESTATION				
MEAN (*)	21.5	21.6	21.9	21.6
ST.DEV	0.51	0.50	0.34	0.60
N	23	20	23	21
IMPLANTATIONS				
TOTAL	303	248	301	258
MEAN (*)	13.2	12.4	13.1	11.7
ST.DEV	2.87	2.28	2.66	2.90
N	23	20	23	22
POST IMPLANTATION LOSS				
% OF IMPLANTATIONS	12.5	10.1	13.6	14.3
LITTERS AFFECTED	19	15	20	18
TOTAL	38	25	41	37
MEAN (*)	1.7	1.3	1.8	1.7
ST.DEV	1.50	0.91	1.24	1.46
N	23	20	23	22
DEAD PUPS AT FIRST LITTER CHECK				
LITTERS AFFECTED	2	1	1	1
TOTAL	2	1	1	1
MEAN (*)	0.1	0.1	0.0	0.0
ST.DEV	0.29	0.22	0.21	0.21
N	23	20	23	22
LIVING PUPS AT FIRST LITTER CHECK				
% OF MALES / FEMALES	48 / 52	52 / 48	45 / 55	44 / 56
TOTAL	265	223	260	221
MEAN (*)	11.5	11.2	11.3	10.0
ST.DEV	3.12	2.16	2.93	2.80
N	23	20	23	22
POSTNATAL LOSS DAYS 0 - 4 P.P.				
% OF LIVING PUPS	1.9	0.4	0.4	1.4
LITTERS AFFECTED	5	1	1	3
TOTAL	5	1	1	3
MEAN (*)	0.2	0.1	0.0	0.1
ST.DEV	0.42	0.22	0.21	0.35
N	23	20	23	22
LIVING PUPS DAY 4 P.P.				
TOTAL	178	158	175	163
MEAN (*)	7.7	7.9	7.6	7.4
ST.DEV	0.86	0.45	1.31	1.40
N	23	20	23	22
BREEDING LOSS DAYS 5 - 21 P.P.				
% OF LIVING PUPS AT DAY 4 P.P.	0.6	0.0	0.6	0.6
LITTERS AFFECTED	1	0	1	1
TOTAL	1	0	1	1
MEAN (*)	0.0	0.0	0.0	0.0
ST.DEV	0.21	0.00	0.21	0.21
N	23	20	23	22
LIVING PUPS DAY 21 P.P.				
% OF MALES / FEMALES	50 / 50	49 / 51	45 / 55	48 / 52

	GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
TOTAL	177	158	174	162
MEAN (*)	7.7	7.9	7.6	7.4
ST.DEV	0.88	0.45	1.31	1.40
N	23	20	23	22

* / ** : Dunnett-test significant at 5% (*) or 1% (**) level

8. Parental post-mortem results

a) Organ weights: In the males at 600 ppm, the weight of the liver adjusted for body weight was statistically significantly higher than control. This effect was considered to be a test item-related effect. No other test article-related findings were observed. In the females, there were no statistically significant differences to the control group for the organ weight adjusted for body weight. Statistically lower absolute weights in the 100 and 250 ppm groups were considered secondary to decreased body weights.

ORGAN WEIGHTS (GRAM)

P GENERATION MALES

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 600 PPM
BODY W.	MEAN	462.3	446.1	449.9	438.6
	ST.DEV.	28.0	48.3	46.6	39.4
	N	25	25	25	25
BRAIN	MEAN	2.02	2.04	2.02	2.03
	ST.DEV.	0.11	0.08	0.08	0.09
	N	25	25	25	25
PITUITARY	MEAN	0.009	0.009	0.009	0.009
	ST.DEV.	0.001	0.001	0.002	0.001
	N	25	25	25	25
THYROID(R)	MEAN	0.012	0.013	0.013	0.014
	ST.DEV.	0.003	0.004	0.004	0.004
	N	25	25	25	25
THYROID(L)	MEAN	0.013	0.012	0.013	0.013
	ST.DEV.	0.003	0.003	0.003	0.003
	N	25	25	25	25
LIVER	MEAN	12.55	12.42	12.26	13.50
	ST.DEV.	1.01	1.51	1.81	1.34
	N	25	25	25	25
KIDNEY(R)	MEAN	1.17	1.12	1.10	1.09 *
	ST.DEV.	0.10	0.11	0.14	0.11
	N	25	25	25	25
KIDNEY(L)	MEAN	1.13	1.11	1.08	1.08
	ST.DEV.	0.10	0.10	0.11	0.11
	N	25	25	25	25
SPLEEN	MEAN	0.88	0.84	0.85	0.80 *
	ST.DEV.	0.14	0.10	0.14	0.09
	N	25	25	25	25
ADRENAL(L)	MEAN	0.030	0.028	0.028	0.027
	ST.DEV.	0.005	0.004	0.004	0.004
	N	25	25	25	25
ADRENAL(R)	MEAN	0.028	0.025 *	0.026	0.026
	ST.DEV.	0.004	0.003	0.004	0.004
	N	25	25	25	25
TESTIS(L)	MEAN	1.97	2.01	1.91	1.97
	ST.DEV.	0.14	0.26	0.28	0.17
	N	25	25	25	25
TESTIS(R)	MEAN	1.94	2.00	1.90	1.93
	ST.DEV.	0.12	0.28	0.27	0.18
	N	25	25	25	25
PROSTATE	MEAN	0.98	0.98	0.99	1.00
	ST.DEV.	0.15	0.16	0.14	0.12
	N	25	25	25	25
EPIDIDI(R)	MEAN	0.797	0.769	0.723 **	0.752
	ST.DEV.	0.069	0.089	0.081	0.079
	N	25	25	25	25
EPIDIDI(L)	MEAN	0.603	0.577	0.560	0.582
	ST.DEV.	0.046	0.066	0.076	0.071
	N	25	25	25	25
EPIDCAUD(R)	MEAN	0.298	0.297	0.282	0.294
	ST.DEV.	0.030	0.035	0.045	0.027
	N	25	25	25	25
SEMINAL VE	MEAN	1.43	1.26	1.32	1.26
	ST.DEV.	0.29	0.24	0.30	0.22
	N	25	25	25	25

*/**: Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level.

ORGAN WEIGHT ADJUSTED FOR BODY WEIGHT (GRAM)

P GENERATION MALES

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 600 PPM
BRAIN	MEAN	2.02	2.04	2.02	2.03
	ST.DEV.	0.11	0.08	0.09	0.09
	N	25	25	25	25
PITUITARY	MEAN	0.009	0.009	0.009	0.010
	ST.DEV.	0.001	0.001	0.002	0.001
	N	25	25	25	25
THYROID(R)	MEAN	0.012	0.013	0.013	0.014
	ST.DEV.	0.003	0.004	0.004	0.004
	N	25	25	25	25
THYROID(L)	MEAN	0.013	0.012	0.013	0.013
	ST.DEV.	0.003	0.003	0.003	0.003
	N	25	25	25	25
LIVER	MEAN	12.18	12.51	12.24	13.79 **
	ST.DEV.	1.01	1.51	1.81	1.34
	N	25	25	25	25
KIDNEY(R)	MEAN	1.15	1.13	1.11	1.11
	ST.DEV.	0.11	0.11	0.14	0.11
	N	25	25	25	25
KIDNEY(L)	MEAN	1.11	1.12	1.08	1.10
	ST.DEV.	0.10	0.10	0.11	0.11
	N	25	25	25	25
SPLEEN	MEAN	0.87	0.85	0.85	0.81
	ST.DEV.	0.14	0.10	0.14	0.09
	N	25	25	25	25
ADRENAL(L)	MEAN	0.030	0.028	0.028	0.028
	ST.DEV.	0.005	0.004	0.004	0.004
	N	25	25	25	25
ADRENAL(R)	MEAN	0.028	0.025	0.026	0.026
	ST.DEV.	0.004	0.003	0.004	0.004
	N	25	25	25	25
TESTIS(L)	MEAN	1.95	2.02	1.91	1.98
	ST.DEV.	0.14	0.26	0.28	0.17
	N	25	25	25	25
TESTIS(R)	MEAN	1.92	2.00	1.90	1.94
	ST.DEV.	0.13	0.28	0.27	0.18
	N	25	25	25	25
PROSTATE	MEAN	0.97	0.98	0.99	1.01
	ST.DEV.	0.15	0.16	0.14	0.12
	N	25	25	25	25
EPIDIDI(R)	MEAN	0.790	0.771	0.723 **	0.757
	ST.DEV.	0.069	0.089	0.081	0.079
	N	25	25	25	25
EPIDIDI(L)	MEAN	0.598	0.578	0.560	0.586
	ST.DEV.	0.046	0.066	0.076	0.071
	N	25	25	25	25
EPIDCAUD(R)	MEAN	0.295	0.298	0.282	0.297
	ST.DEV.	0.030	0.035	0.045	0.027
	N	25	25	25	25
SEMINAL VE	MEAN	1.42	1.27	1.32	1.27
	ST.DEV.	0.29	0.24	0.30	0.22
	N	25	25	25	25

*/**: Dunnett-test (with Anacova) based on pooled variance significant at 5% (*) or 1% (**) level

ORGAN WEIGHTS (GRAM)
P GENERATION FEMALES

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
BODY W.	MEAN	291.4	284.4	275.5 **	255.7 **
	ST.DEV.	13.6	20.7	11.3	23.5
	N	23	20	23	22
BRAIN	MEAN	1.88	1.86	1.85	1.84
	ST.DEV.	0.08	0.09	0.08	0.08
	N	23	20	23	22
PITUITARY	MEAN	0.014	0.013	0.014	0.014
	ST.DEV.	0.002	0.002	0.002	0.002
	N	23	20	23	22
THYROID(R)	MEAN	0.011	0.010	0.011	0.011
	ST.DEV.	0.003	0.002	0.002	0.003
	N	23	20	23	22
THYROID(L)	MEAN	0.011	0.010	0.011	0.011
	ST.DEV.	0.002	0.002	0.003	0.003
	N	23	20	23	22
LIVER	MEAN	12.70	12.69	11.65 *	11.15 **
	ST.DEV.	1.22	1.23	1.29	1.67
	N	23	20	23	22
KIDNEY(R)	MEAN	0.92	0.93	0.90	0.85 **
	ST.DEV.	0.07	0.07	0.06	0.08
	N	23	20	23	22
KIDNEY(L)	MEAN	0.90	0.90	0.88	0.83 **
	ST.DEV.	0.08	0.09	0.06	0.07
	N	23	20	23	22
SPLEEN	MEAN	0.79	0.78	0.73	0.65 **
	ST.DEV.	0.11	0.12	0.10	0.15
	N	23	20	23	22
ADRENAL(L)	MEAN	0.042	0.044	0.042	0.042
	ST.DEV.	0.004	0.007	0.005	0.006
	N	23	20	23	22
ADRENAL(R)	MEAN	0.039	0.039	0.039	0.036
	ST.DEV.	0.006	0.006	0.006	0.006
	N	23	20	23	22
OVARIES(L)	MEAN	0.058	0.056	0.054	0.049 *
	ST.DEV.	0.012	0.010	0.009	0.011
	N	23	20	23	22
OVARIES(R)	MEAN	0.053	0.053	0.051	0.047
	ST.DEV.	0.010	0.010	0.009	0.011
	N	23	20	23	22
UTERUS	MEAN	0.80	0.81	0.75	0.78
	ST.DEV.	0.15	0.15	0.12	0.20
	N	23	20	23	22

*/**: Dunnett-Test based on pooled variance significant at 5% (*) or 1% (**) level.

ORGAN WEIGHT ADJUSTED FOR BODY WEIGHT (GRAM)
P GENERATION FEMALES

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
BRAIN	MEAN	1.85	1.84	1.86	1.88
	ST.DEV.	0.08	0.09	0.08	0.08
	N	23	20	23	22
PITUITARY	MEAN	0.014	0.013	0.014	0.014
	ST.DEV.	0.002	0.002	0.002	0.002
	N	23	20	23	22
THYROID(R)	MEAN	0.011	0.010	0.011	0.011
	ST.DEV.	0.003	0.002	0.002	0.003
	N	23	20	23	22
THYROID(L)	MEAN	0.010	0.010	0.011	0.012
	ST.DEV.	0.002	0.002	0.003	0.003
	N	23	20	23	22
LIVER	MEAN	11.91	12.28	11.72	12.29
	ST.DEV.	1.23	1.23	1.29	1.67
	N	23	20	23	22
KIDNEY(R)	MEAN	0.89	0.91	0.90	0.90
	ST.DEV.	0.07	0.07	0.06	0.08
	N	23	20	23	22
KIDNEY(L)	MEAN	0.97	0.88	0.89	0.89
	ST.DEV.	0.08	0.09	0.06	0.07
	N	23	20	23	22
SPLEEN	MEAN	0.75	0.76	0.74	0.72
	ST.DEV.	0.11	0.12	0.10	0.15
	N	23	20	23	22
ADRENAL(L)	MEAN	0.040	0.043	0.043	0.044
	ST.DEV.	0.004	0.007	0.005	0.006
	N	23	20	23	22
ADRENAL(R)	MEAN	0.038	0.038	0.039	0.038
	ST.DEV.	0.006	0.006	0.006	0.006
	N	23	20	23	22
OVARIES(L)	MEAN	0.055	0.054	0.054	0.053
	ST.DEV.	0.012	0.010	0.009	0.011
	N	23	20	23	22
OVARIES(R)	MEAN	0.050	0.052	0.052	0.051
	ST.DEV.	0.010	0.010	0.009	0.011
	N	23	20	23	22
UTERUS	MEAN	0.80	0.81	0.75	0.78
	ST.DEV.	0.15	0.15	0.12	0.20
	N	23	20	23	22

*/**: Dunnett-test (with Anacova) based on pooled variance significant at 5% (*) or 1% (**) level.

b) Macroscopic examination: No test item-related findings were observed in males or females at any dose level at necropsy. It was confirmed at necropsy that one female in the 25 ppm dose group, which was killed for ethical reasons, had skin lesions. No other macroscopical findings were observed in this female. The cause of the death of one female in the 250 ppm group could not be established.

c) Microscopic examination: In both P and F1 generations, the males in the 600 ppm dose group, the incidence of centrilobular hepatocellular hypertrophy was increased. In the 100 and 250 ppm group females, the incidence of hepatocellular glycogen deposits was decreased compared to the control group. Based on vaginal micropathology to determine estrous cycle status at the end of lactation, an increased number of animals in lactational diestrus was observed at 250 ppm (10/25) when compared to controls (1/25).

At 100 ppm the incidence was slightly increased (3/25) when compared to controls, however, this was within normal range for animals of this strain and age. There was no difference at 25 ppm.

Quantitation of ovarian follicles: The follicle count made during micropathological examination of ovarian tissues from females of the F1 generation, showed a lower number of growing follicles by a large magnitude of 34.4% reductions in the high dose group as compared to the control group, with statistical significance. However, the Agency does not consider this effect to be adverse since the antral follicle counts in the later stage of development were normal. The follicle counts for the 25 and 100 ppm groups were not evaluated.

	Group	Follicles			Corpora lutea
		primordial	growing	antral	
Total /group	**Group 1**	1886	192	63	178
		2078			
	Group 4	1591	126	66	116
		1717			
Mean /animal	**Group 1**	188.60	19.20	6.30	17.80
		207.80			
	Group 4	159.10	12.60	6.60	11.60
		171.70			
SD /animal	**Group 1**	74.00	6.56	3.13	5.41
		79.05			
	Group 4	82.29	3.41	1.78	2.84
		81.09			
Min /animal	**Group 1**	70	9	2	10
		79			
	Group 4	53	8	5	8
		67			
Max /animal	**Group 1**	336	29	12	28
		365			
	Group 4	342	18	10	16
		353			
Median /animal	**Group 1**	182.00	18.50	5.50	17.00
		203.00			
	Group 4	152.00	12.50	6.00	10.50
		168.00			
P-Value (Wilcoxon)	**4 vs. 1**	0.36	0.03	0.60	0.012
		0.29			
P-Value ($\chi 2$)	**4 vs. 1**	0.36	0.012*	0.62	0.003**
		0.30			

* P-value F-test <0.05, ** P-value F-test <0.01

B. Offspring:

1. Viability: Survival was not affected by treatment with the test article. Eight pups died or were lost in the control group compared to 2 in the 25 ppm group, 3 in the 100 ppm group and 9 in the 250 ppm group.

BREEDING DATA PER GROUP
F1 GENERATION

	GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
LITTERS				
TOTAL	23	25	24	25
DURATION OF GESTATION				
MEAN (*)	21.5	21.7	21.5	21.5
ST.DEV	0.51	0.48	0.51	0.51
N	23	24	24	25
IMPLANTATIONS				
TOTAL	293	317	308	309
MEAN (*)	12.7	12.7	12.8	12.4
ST.DEV	2.22	2.21	1.58	2.45
N	23	25	24	25
POST IMPLANTATION LOSS				
% OF IMPLANTATIONS	10.2	7.6	5.2	10.7
LITTERS AFFECTED	16	17	10	16
TOTAL	30	24	16	33
MEAN (*)	1.3	1.0	0.7	1.3
ST.DEV	2.05	0.84	1.01	1.75
N	23	25	24	25
DEAD PUPS AT FIRST LITTER CHECK				
LITTERS AFFECTED	0	0	0	2
TOTAL	0	0	0	4
MEAN (*)	0.0	0.0	0.0	0.2
ST.DEV	0.00	0.00	0.00	0.62
N	23	25	24	25
LIVING PUPS AT FIRST LITTER CHECK				
% OF MALES / FEMALES	44 / 56	47 / 53	53 / 47	47 / 53
TOTAL	263	293	292	276
MEAN (*)	11.4	11.7	12.2	11.0
ST.DEV	2.71	2.37	1.69	3.03
N	23	25	24	25
POSTNATAL LOSS DAYS 0 - 4 P.P.				
% OF LIVING PUPS	0.4	0.0	0.3	1.4
LITTERS AFFECTED	1	0	1	4
TOTAL	1	0	1	4
MEAN (*)	0.0	0.0	0.0	0.2
ST.DEV	0.21	0.00	0.20	0.37
N	23	25	24	25
LIVING PUPS DAY 4 P.P.				
TOTAL	179	197	192	189
MEAN (*)	7.8	7.9	8.0	7.6
ST.DEV	0.85	0.60	0.00	1.53
N	23	25	24	25
BREEDING LOSS DAYS 5 - 21 P.P.				
% OF LIVING PUPS AT DAY 4 P.P.	1.7	0.0	0.0	0.5
LITTERS AFFECTED	1	0	0	1
TOTAL	3	0	0	1
MEAN (*)	0.1	0.0	0.0	0.0
ST.DEV	0.63	0.00	0.00	0.20
N	23	25	24	25
LIVING PUPS DAY 21 P.P.				
% OF MALES / FEMALES	44 / 56	47 / 53	51 / 49	51 / 49
TOTAL	176	197	192	188
MEAN (*)	7.7	7.9	8.0	7.5
ST.DEV	1.03	0.60	0.00	1.53
N	23	25	24	25

* / ** : Dunnett-test significant at 5% (*) or 1% (**) level

BREEDING DATA PER GROUP
F1 GENERATION

	GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
BIRTH INDEX (*)	89.8	92.4	94.8	89.3
VIABILITY INDEX (*)	99.6	100.0	99.7	98.6
WEANING INDEX (*)	98.3	100.0	100.0	99.5

2. Clinical signs: At the first litter check, no test item-related findings were observed. One female pup in the 25 ppm group had an injury to the inguinal region and a male pup had a bite wound on the abdomen. In the 250 ppm group, 4 pups in litter 178, which were found dead at the first litter check, had no milk in the stomach. No other findings were observed in any pup in any dose group. During the lactation period, no test item-related clinical signs were observed. One male pup in the control group had a bite wound. In the 25 ppm group, wounds or injuries were observed in 3 pups in 3 separate litters. In the 250 ppm group, irregular fur growth was observed in the pups in litter 183. No other findings were observed in any pups in any dose group.

3. Body weight: On day 1 post partum, mean body weight of the pups was similar in all dose groups. The body weight of the pups in the 250 ppm dose group was statistically significantly lower than control from day 7 of the lactation period (female pups), or from day 14 of the lactation period (male pups) onwards. This was considered to be a test item-related effect. In the 25 and 100 ppm dose groups, mean body weight development was similar to that of the control group.

MEAN BODY WEIGHTS OF PUPS PER GROUP (GRAM)
F1 PUPS

DAY	SEX		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
0	M	MEAN	6.1	6.3	6.0	6.3
		ST.DEV.	0.25	0.18	0.24	0.50
		N	5	3	7	5
	F	MEAN	6.1	5.8	5.9	6.2
		ST.DEV.	0.26	0.29	0.30	0.38
		N	5	3	9	5
	M+F	MEAN	6.1	6.1	5.9	6.2
		ST.DEV.	0.25	0.17	0.24	0.41
		N	5	3	9	5
1	M	MEAN	6.4	6.7	6.5	6.4
		ST.DEV.	0.55	0.71	0.50	0.68
		N	22	20	21	22
	F	MEAN	6.3	6.3	6.3	6.1
		ST.DEV.	0.59	0.69	0.48	0.77
		N	23	20	23	21
	M+F	MEAN	6.4	6.5	6.4	6.2
		ST.DEV.	0.57	0.68	0.45	0.71
		N	23	20	23	22
4	M	MEAN	9.9	10.3	9.8	9.8
		ST.DEV.	1.22	1.10	1.04	1.43
		N	22	20	21	22
	F	MEAN	9.7	9.9	9.7	9.4
		ST.DEV.	1.40	1.16	1.02	1.45
		N	23	20	23	21
	M+F	MEAN	9.8	10.1	9.7	9.6
		ST.DEV.	1.34	1.09	1.00	1.43
		N	23	20	23	22
7	M	MEAN	16.5	17.0	16.4	15.6
		ST.DEV.	1.74	1.44	1.40	1.85
		N	22	20	21	22
	F	MEAN	16.4	16.4	15.8	15.1 *
		ST.DEV.	1.89	1.53	1.46	1.80
		N	23	20	23	21
	M+F	MEAN	16.5	16.7	15.9	15.4
		ST.DEV.	1.81	1.44	1.49	1.78
		N	23	20	23	22
14	M	MEAN	34.1	34.0	33.7	30.7 **
		ST.DEV.	2.65	2.34	2.21	3.04
		N	22	20	21	22
	F	MEAN	33.8	33.2	32.9	30.3 **
		ST.DEV.	2.72	2.53	2.35	2.94
		N	23	20	23	21
	M+F	MEAN	34.0	33.7	33.1	30.5 **
		ST.DEV.	2.64	2.37	2.45	2.88
		N	23	20	23	22
21	M	MEAN	52.4	52.6	53.1	47.0 **
		ST.DEV.	4.04	3.42	3.43	4.63
		N	22	20	21	22

21	F	MEAN	51.9	51.4	51.6	45.8 **
		ST.DEV.	3.82	3.60	3.63	4.51
		N	23	20	23	21
	M+F	MEAN	52.3	52.0	52.0	46.4 **
		ST.DEV.	3.80	3.43	3.86	4.39
		N	23	20	23	22

* / ** : Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

3. <u>Sexual maturation (F_1 generation)</u>: The time until preputial separation was statistically significantly longer in the males in the 600 ppm dose group, however, the body weight at the time of sexual maturation was similar to the control value and other treated group values. This delay in sexual maturation in selected F1 males at 250 ppm is considered to reflect the lower body weight of these animals rather than a direct effect of SYN545192. The time until vaginal patency in the F1 female pups was not affected by treatment with the test article.

SEXUAL MATURATION
PREPUTIAL SEPARATION
F1 GENERATION MALES

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 600 PPM
Day p.p.	MEAN	26.5	26.2	26.8	28.4 **
	ST.DEV.	1.4	1.0	1.4	1.9
	N	25	25	25	25
Weight (g)	MEAN	77.15	76.86	80.20	79.27
	ST.DEV.	11.06	5.57	9.47	11.33
	N	25	25	25	25

SEXUAL MATURATION
VAGINAL PATENCY
F1 GENERATION FEMALES

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
Day p.p.	MEAN	33.3	33.6	33.5	34.6 *
	ST.DEV.	1.6	1.9	1.7	2.4
	N	25	25	24	25
Weight (g)	MEAN	106.22	108.84	108.81	103.87
	ST.DEV.	12.20	12.92	10.02	11.31
	N	25	25	24	25

4. <u>Anogenital distance (F_2 pups)</u>: The anogenital distance was not affected by treatment with the test item.

5. <u>Offspring postmortem results</u>:

a) Organ weights: In the 250 ppm F1 males, the weight of the spleen adjusted for the body weight was statistically significantly reduced. In addition, the weight of the liver adjusted for body weight was statistically significantly increased. In the F2 male pups, the organ/body weight ratio of the brain was statistically significantly increased. In the 250 ppm females in both generations, the weight of the liver adjusted for body weight was statistically significantly increased.

ORGAN WEIGHTS (GRAM)
F1 PUPS MALES

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
BODY W.	MEAN	51.5	50.8	51.9	45.4 **
	ST.DEV.	4.3	4.0	3.8	3.7
	N	22	20	20	21
BRAIN	MEAN	1.48	1.48	1.49	1.49
	ST.DEV.	0.06	0.08	0.06	0.06
	N	22	20	20	21
LIVER	MEAN	1.93	1.91	2.01	1.80
	ST.DEV.	0.25	0.17	0.23	0.20
	N	22	20	20	21
THYMUS	MEAN	0.201	0.198	0.210	0.185
	ST.DEV.	0.038	0.037	0.035	0.032
	N	22	20	20	21
SPLEEN	MEAN	0.26	0.25	0.25	0.18 **
	ST.DEV.	0.04	0.05	0.05	0.03
	N	22	20	20	21

ORGAN WEIGHT ADJUSTED FOR BODY WEIGHT (GRAM)
F1 PUPS MALES

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
BRAIN	MEAN	1.46	1.47	1.47	1.53 **
	ST.DEV.	0.06	0.08	0.06	0.06
	N	22	20	20	21
LIVER	MEAN	1.86	1.87	1.92	2.00 **
	ST.DEV.	0.25	0.17	0.23	0.20
	N	22	20	20	21
THYMUS	MEAN	0.193	0.193	0.199	0.209
	ST.DEV.	0.038	0.037	0.035	0.032
	N	22	20	20	21
SPLEEN	MEAN	0.25	0.24	0.23	0.21 **
	ST.DEV.	0.04	0.05	0.05	0.03
	N	22	20	20	21

ORGAN WEIGHTS (GRAM)
F1 PUPS FEMALES

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
BODY W.	MEAN	49.9	49.0	49.9	44.4 **
	ST.DEV.	4.4	3.8	3.8	4.6
	N	21	20	23	21
BRAIN	MEAN	1.44	1.43	1.45	1.43
	ST.DEV.	0.05	0.06	0.04	0.07
	N	21	20	23	21
LIVER	MEAN	1.80	1.76	1.85	1.65
	ST.DEV.	0.21	0.19	0.20	0.21
	N	21	20	23	21
THYMUS	MEAN	0.218	0.206	0.207	0.183 **
	ST.DEV.	0.038	0.040	0.033	0.028
	N	21	20	23	21
SPLEEN	MEAN	0.25	0.25	0.24	0.19 **
	ST.DEV.	0.05	0.04	0.06	0.04
	N	21	20	23	21

ORGAN WEIGHT ADJUSTED FOR BODY WEIGHT (GRAM)
F1 PUPS FEMALES

		GROUP 1 0 PPM	GROUP 2 25 PPM	GROUP 3 100 PPM	GROUP 4 250 PPM
BRAIN	MEAN	1.43	1.43	1.44	1.46
	ST.DEV.	0.05	0.06	0.04	0.07
	N	21	20	23	21
LIVER	MEAN	1.73	1.74	1.78	1.82 *
	ST.DEV.	0.21	0.19	0.20	0.21
	N	21	20	23	21
THYMUS	MEAN	0.210	0.203	0.199	0.203
	ST.DEV.	0.038	0.040	0.033	0.028
	N	21	20	23	21
SPLEEN	MEAN	0.24	0.25	0.23	0.22
	ST.DEV.	0.05	0.04	0.06	0.04
	N	21	20	23	21

b) Macroscopic examination: No test item-related macroscopical findings were noted at necropsy at any dose level in the F1 or F2 pups.

c) Microscopic examination: Additional findings observed in F1 generation were an increased incidence of hypertrophy of adrenal zona glomerulosa in females at 250 ppm, an increased incidence of patchy fatty change in the liver of males at all dose levels, and an increased incidence of cell hypertrophy in the pars distalis of pituitary in males at 600 ppm. In the 250 ppm F1 group, the mean corpora lutea count at necropsy was decreased by 7.5%, however without statistical significance.

Incidence and Mean Grade of Patchy Fatty Change in the Liver, F1 Generation

Dose level ppm	0		25		100		600	250
Sex	M	F	M	F	M	F	M	F
Incidence	1/25	0/25	5/25	0/25	8/25	0/25	10/25	0/25
Mean grade	1.0		1.2		1.1		1.0	

Hepatocytic glycogen deposits had decreased incidence and mean grade in female P generation, and decreased incidence in F1 generation females at 100 and 250 ppm.

Incidence and Mean Grade of Hepatocytic Glycogen Deposits, P Generation

Dose level ppm	0		25		100		600	250
Sex	M	F	M	F	M	F	M	F
Incidence	16/25	19/25	19/25	19/25	18/25	5/25	20/25	7/25
Mean grade	1.2	1.2	1.2	1.1	1.0	1.0	1.1	1.0

Incidence and Mean Grade of Hepatocytic Glycogen Deposits, F1 Generation

Dose level ppm	0		25		100		600	250
Sex	M	F	M	F	M	F	M	F
Incidence	11/25	21/25	11/25	20/25	16/25	14/25	10/25	8/25
Mean grade	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0

Incidence and Mean Grade of Pituitary Pars Distalis Hypertrophy, F1 Generation

Dose level ppm	0		25		100		600	250
Sex	M	F	M	F	M	F	M	F
Incidence	9/25	0/25	11/25	0/25	15/25	0/25	16/25	0/25
Mean grade	1.2		1.0		1.0		1.3	

III. DISCUSSION

1. Investigator conclusions (extracted from page 66 in the study report) –

SYN545192 was administered orally, by ingestion, continuously throughout the study to males over a pre-pairing and pairing period up to one day before necropsy and to females over the pre-pairing, pairing, gestation and lactation period at dose levels of 0, 25, 100 and 250 (females) or 600 ppm (males). In the 600 ppm dose group males and 250 ppm dose group females, there was a reduction in mean food consumption and a reduction in body weight and body weight gain in both generations. In males the liver weight adjusted for body weight was increased and microscopic examination revealed centrilobular hepatocellular hypertrophy in both generations. There was also an increased incidence of

cell hypertrophy in the pars distalis of the pituitary in the F1 generation. In females liver weight adjusted for body weight was increased in the F1 generation only and there were no adverse micropathology changes. In the pups at 250 ppm, body weight was reduced in both generations. In F1 male pups and F1 and F2 female pups, the weight of the liver adjusted for body weight was statistically significantly increased. In F1 male pups, the weight of the spleen adjusted for the body weight was statistically significantly reduced. A higher number of dams were in "lactational diestrus" at day 21 of lactation, which was considered an indirect consequence of high dose effects on pup and maternal body weight, and not a direct effect of treatment. The time until preputial separation in F1 male pups was increased, and this was considered to reflect the lower body weight of these animals.

In the 100 ppm group, body weight in the P generation females was slightly reduced towards the end of the pre-pairing period and was occasionally reduced during the gestation and lactation periods. The lack of similar effects in the F1 generation females indicates these differences were not adverse. In the 25 ppm group, no test item-related findings were noted in either generation during the study. SYS545192 had no effect on any parameter of reproduction across two generations at dose levels up to 600 ppm in males (equivalent to 40.5 mg/kg/day for P generation males during pre-pairing) and 250 ppm in females (equivalent to 19.4 mg/kg/day for P generation females during pre-pairing). Based on the results of this study, the parental NOAEL (No Observed Adverse Effect Level) was considered to be 100 ppm and the offspring NOEL (No Observed Effect Level) for general toxic effects was 100 ppm. The NOEL for reproduction was 250 ppm in females and 600 ppm in males.

2. <u>Reviewer conclusions</u> - The Agency concurs with the NOAEL and LOAEL selections for the parents, reproduction and offspring. The growing follicle counts at 250 ppm were decreased by 34.4% and the 25 and 100 ppm groups were not tested for follicle counts. However, the antral follicle counts in a later stage of development were normal, so the Agency does not consider the decrease in growing follicle counts to be adverse. However, the Agency does not concur that the effect of the test article on follicle counts or corpora lutea counts or lactational diestrus was via excessive decreases in dam and pup body weights. The female parental body weight was decreased by an average of 4.0% during the 70-day pre-pairing period at the top dose, not considered adverse nor large nor excessive weight loss. The female parental body weight was decreased by an average of 11.0% during the gestation period at the top dose, considered adverse but not large nor excessive. The female parental body weight during lactation was decreased by an average of 11.8% at the top dose, considered adverse but not large nor excessive. The time-based composite decrease in body weight for the parental females during the entire study was a 6.8% reduction. In the parental females, the reduced food consumption during these periods at the high dose were similar magnitudes of 0-21.0% reductions and may explain the decreased body weights. The decreased glycogen in the liver and decreased follicle counts could be due to inhibition of mitochondrial function via the test article, its pesticidal mode of action. Decreased glycogen content is consistent with increased glycolytic metabolism during oxidative metabolic inhibition. Follicles contain more

mitochondria than any tissue, thus the effect of mitochondrial inhibition is consistent with the effects measured.

The parental NOAEL (No Observed Adverse Effect Level) was 100 ppm (equivalent to 6.8 mg/kg/day for P generation males and 8.2 mg/kg/day during pre-pairing). The parental LOAEL is 600 ppm in males (40.5 mg/kg/day) and 250 ppm in females (19.4 mg/kg/day) due to decreased body weight, decreased body weight gain, and decreased food consumption.

The offspring NOAEL for general toxic effects was 100 ppm (equivalent to 7.8 mg/kg/day for F1 generation males and 8.7 mg/kg/day during pre-pairing). The offspring LOAEL is 600 ppm or 48.0 mg/kg/day for males and 250 ppm or 22.0 mg/kg/day for females. In males the liver weight adjusted for body weight was increased and microscopic examination revealed centrilobular hepatocellular hypertrophy in both generations. There was also an increased incidence of cell hypertrophy in the pars distalis of the pituitary in the F1 generation. In females liver weight adjusted for body weight was increased in the F1 generation only and there were no adverse micropathology changes. In the pups at 250 ppm, body weight was reduced in both generations. In F1 male pups and F1 and F2 female pups, the weight of the liver adjusted for body weight was statistically significantly increased. In F1 male pups, the weight of the spleen adjusted for the body weight was statistically significantly reduced. A higher number of dams were in "lactational diestrus" at day 21 of lactation.The time until preputial separation in F1 male pups was increased.

The NOAEL for reproduction was 600 ppm in males or 40.5 mg/kg/day for P generation males during pre-pairing and 250 ppm or 19.4 mg/kg/day in females. The LOAEL for reproduction in both genders was not determined, since the NOAEL was the HDT.

3. Deficiencies - Some of the methods were not described in detail. This minor deficiency is not expected to affect the outcome of this study. The low and mid dose follicle counts were not provided, thus a NOAEL for this endpoint was not determined.

4. References -

1. E. Salewski: Arch. Exp. Path. Pharmak. 247, pp. 367-368 (1964)

2. C.W. Dunnett: A Multiple Comparison Procedure for Comparing Several Treatments with a Control, J. Amer. Statist. Assoc. 50, pp. 1096-1121 (1955).

3. R.A. Fisher: Statistical Methods for Research Workers, Oliver and Boyd, Edinburgh (1950).

4. H. Tsukamura and K. Maeda. Non-metabolic and metabolic factors causing lactational anestrus: rat models uncovering the neuroendocrine mechanism underlying the suckling-induced changes in the mother. Chapter 13 In: Progress in Brain Research, Vol. 133. (2001).

Report:	IIA 5.6.10/02 Whitlow S. (2011). **SYN545192 - Prenatal Developmental Toxicity Study in the Han Wistar Rat** Harlan Laboratories Ltd. Wölferstrasse 4 4414 Füllinsdorf, Switzerland, Laboratory Report No. C73670. Final Report Issue date: September 15, 2011. Unpublished. MRID # 48604451

Guidelines: Prenatal Developmental Toxicity Study (rat) adapted from OPPTS 870.3700 (1998): OECD 414 (2001): 2004/73/EC B.31 (2004): JMAFF, 12 Nohsan No. 8147 (2000)

Sponsor: Syngenta Crop Protection, LLC 410 Swing Road, Post Office Box 18300 Greensboro, NC 27419-8300 USA

Executive Summary: In this prenatal developmental toxicity study the doses of 7.5, 15, and 30 mg/kg body weight/day were administered orally, by gavage, to pregnant Han Wistar rats from day 6 to 20 post coitum.

The dose level of 30 mg/kg body weight/day produced obvious maternal toxicity. Clinical signs of decreased activity, hunched posture, ataxia and ruffled fur were noted during the treatment period. Food consumption, body weight and corrected body weight gains were statistically significantly reduced. Fetal weights were also statistically significantly reduced in this dose group and at skeletal examination a slight delay in ossification was observed. No test item-related findings were found during the study in the 7.5 and 15 mg/kg body weight/ day dose groups.

The NOAEL (No-Observed-Adverse Effect-Level) for maternal effects was 15 mg/kg body weight/day, based on clinical signs (ataxia, decreased activity, hunched posture, ruffled fur) and decreased food consumption at the maternal LOAEL (Lowest Observed Adverse Effect Level) of 30 mg/kg/day. The NOAEL for developmental toxicity was 15 mg/kg body weight/day based on decreased fetal weight and ossification at the developmental LOAEL of 30 mg/kg body weight/day. No biologically significant morphological alterations, including teratogenicity, were observed in this study.

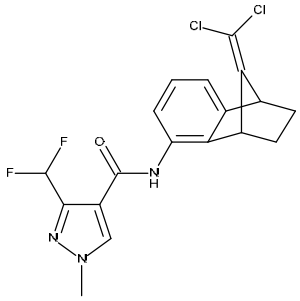
The developmental toxicity study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

Compliance: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No claim of CBI was made for any information contained in this document on the basis of the definition of CBI.

I. MATERIALS AND METHODS

A. Materials:

Test Material: SYN545192



Description:	beige powder
Lot/Batch number:	SMU9BP005
Purity / Composition:	97.0%
Stability of test compound:	8 days refrigerated (5 ± 3 °C); based on Harlan Laboratories study C80036 10 days stability refrigerated (5 ± 3 °C) was also proved within the frame of this study Expiry date: 28-Feb-2013
Vehicle:	0.5% CMC high viscosity (Fluka, lot 1340439)
Positive control:	None

Test Animals:

Species	Rats
Strain	RccHanTM: WIST(SPF)
Age at day 0 p.c.	At least 11 weeks
Weight at day 0 p.c.	182 to 225 g
Source	Harlan Laboratories, B.V. Kreuzelweg 53 5961 NM Horst, Netherlands
Housing	Animals were housed individually in Makrolon type-3 cages with wire mesh tops and sterilized standard softwood bedding with paper enrichment
Acclimatization period	At least 5 days
Diet	Pelleted standard Kliba-Nafag 3433 rodent maintenance diet (Provimi Kliba AG, 4303 Kaiseraugst, Switzerland) was available *ad libitum* (batch no. 42/10).
Water	Community tap-water from Füllinsdorf was available *ad libitum* in water bottles.
Environmental conditions	Temperature: 22 ± 3 °C Humidity: 30-70 % Air changes: 10-15 / h Photoperiod: 12 hours light and 12 hours dark Music: Background music played at a centrally defined low volume during the light period.

B. Study Design and Methods:

1. In-life dates: Start: 21-Sept-2010 End: 13-April-2011

2. Mating procedure: After acclimatization (minimum of 5 days), females were housed with sexually mature males (1:1) in special automatic mating cages i.e. with synchronized timing to initiate the nightly mating period, until evidence of copulation was observed. This system reduced the variation in the copulation times of the different females. The females were removed and housed individually if: the daily vaginal smear was sperm positive, or a copulation plug was observed. The day of mating was designated day 0 post coitum. Male rats of the same source and strain were used only for mating. These male rats are in the possession of Harlan Laboratories and were not considered part of the test system. The fertility of these males had been proven and was continuously monitored.

3. Animal assignment: Mated rats were assigned to the different groups using a computer-generated random algorithm.

Table IIA 5.6.10/02-1. Study design

Dose (mg/kg bw/day)	Group 1 0	Group 2 7.5	Group 3 15	Group 4 30
# Females	24	24	24	24

4. Dose level selection: The dose levels were selected based on a previous non-GLP dose range-finding prenatal developmental study in the Han Wistar Rat, Harlan Laboratories Study C73668, using dose levels of 0, 10, 20 and 30 mg/kg/day.

5. Dose preparation and analysis: The dose formulations were prepared weekly on a weight/weight basis in ten aliquots (60 mL each = 600 mL total) for each group at the Analytical Department of Harlan Laboratories Ltd. Switzerland under the responsibility of Dr. Katja Morgenthal using the test item as supplied by the Sponsor. SYN545192 was weighed into a glass beaker on a tared precision balance and approximately 80% of the vehicle was added (w/v). Using an appropriate homogenizer, a homogeneous suspension was prepared and the remaining vehicle was added. Separate formulations were made for each concentration. Four weekly preparations were made for each group during the study. Due to mating, the daily dosing volume exceeded 60 mL after completion of all matings therefore volume of weekly dose preparation was increased. One aliquot (60 mL) plus 20 mL sub-aliquot from the remaining 3 aliquots were used each day. Correction factor of 1.031 as well as the density of 0.98 g/mL (determined for test formulations comprising a nominal concentration of 1 mg/mL within Harlan Laboratories study C80036 [see References (7)]) was taken into consideration for preparation of the dose formulations. Homogeneity of the dose formulations was maintained using a magnetic stirrer for at least 30 minutes before administration until after dosing. The control group was treated with the vehicle (0.5% Carboxymethylcellulose aqueoussolution high viscosity) only.

The dose formulations were stored in a refrigerator (5 ± 3 °C) in the dark and at room

temperature under ambient light conditions during administration. Based upon the results of stability analyses performed within the Harlan Laboratories study C80036, dose formulations were stable for at least one week. The samples were analyzed by HPLC coupled to an UV detector following an analytical procedure provided by the Sponsor and adapted at Harlan Laboratories.

Results –

Homogeneity Analysis: The homogeneous distribution of SYN545192 in the preparations was approved because single results found did not deviate more than 2.9% (<5%) from the corresponding mean in dose groups 2 and 4. In group 3 the homogeneous distribution deviated more than 5% (6.1%), however, the result was considered to be acceptable, because it deviated only slightly from target.

Stability Analysis: The test article was found to be stable in dose formulations when kept four hours at room temperature and eight and ten days refrigerated due to recoveries which met the variation limit of 10% from the time-zero (homogeneity) mean.

Concentration analysis: SYN545192 in the range of 91.5% to 103.9% and, thus, the required content limit of ±10% with reference to the nominal concentration was met.

The results indicate the accurate use of the test item SYN545192 and 0.5% (w/v) carboxy methylcellulose sodium high viscosity as vehicle during this study. Dose formulations were found to be homogeneously prepared and sufficient formulation stability under storage conditions was approved.

6. Dosage administration: The rats were dosed orally, by gavage once daily from day 6 post coitum through to day 20 post coitum. All animals received a dose volume of 10 mL/kg body weight with a daily adjustment of the individual volume to the actual body weight. Control animals were dosed similarly with the vehicle alone.

C. Observations

1. Maternal observations and evaluations: Observations for viability / mortality were performed twice daily, for clinical signs daily cage-side clinical observations were made (during acclimatization and up to day of necropsy), food consumption was recorded at 3-day intervals (days 0 - 3, 3 - 6, 6 - 9, 9 - 12, 12 - 15, 15 - 18 and 18 - 21 post coitum) and body weights were recorded daily from day 0 until day 21 post coitum.

At the scheduled necropsy on day 21 post coitum, females were sacrificed by CO_2 asphyxiation and the fetuses removed by Caesarean section. Post mortem examination, including gross macroscopic examination of all internal organs
with emphasis on the uterus, uterine contents, corpora lutea count and position of fetuses in the uterus was performed and the data recorded. The uteri (and contents) of all females with live fetuses were weighed during necropsy on day 21 post coitum to enable the calculation of the corrected body weight gain. If no implantation sites were evident, the

uterus was placed in an aqueous solution of ammonium sulfide to accentuate possible hemorrhagic areas of implantation sites.

2. Fetal observations: Fetuses were removed from the uterus, sexed, weighed individually, examined for gross external abnormalities, sacrificed by a subcutaneous injection of sodium pentobarbital and allocated to one of the following procedures:
1. Microdissection technique (sectioning/dissection technique) [see References]. At least one half of the fetuses from each litter was fixed in Bouin's fixative (one fetus per container). They were examined by a combination of serial sections of the head and microdissection of the thorax and abdomen. This included detailed examination of the major blood vessels and sectioning of the heart and kidneys. After examination, the tissue was preserved in a solution of glycerin/ethanol (one fetus per container). Descriptions of any abnormalities and variations were recorded.

2. The remaining fetuses were eviscerated and with the exception of over the paws, the skin was removed and discarded. Carcasses were processed through solutions of ethanol, glacial acetic acid with Alcian blue (for cartilage staining), potassium hydroxide with Alizarin red S (for clearing and staining ossified bone) and aqueous glycerin for preservation and storage. The skeletons were
examined and all abnormal findings and variations were recorded. The specimens were preserved individually in plastic vials.

Fetuses with abnormalities were photographed, when considered appropriate.

D. Data Analysis

1. Statistical analyses: The following statistical methods were used to analyze food consumption, body weight, body weight gain, reproduction and fetal examination data:
 Means and standard deviations of various data were calculated.
 All statistical tests were two-sided.
 Statistical significance between groups was evaluated by Analysis of Variance (ANOVA). In the case where variances were non-homogenous, appropriate transformations were applied (e.g. log, square root, or double arcsine) to stabilize the variances before the ANOVA. The Dunnett many-one t-test [see References (5)] was then used to compare each group to control based on the error mean square in the ANOVA.
 Fisher’s exact-test [see References (6)] was applied if the variables could be dichotomized without loss of information.
 For statistical tests on fetal data, comparisons were made between groups for number of fetuses affected and number of litters affected, for completeness. The litter was considered the proper unit of measurement for overall study evaluation.

2. Indices: The following data were recorded on-line: clinical signs and observations, food consumption, body weights, necropsy findings (RCC-TOX CONTROL LIMS), reproduction data, uterus weights at Caesarean section and skeletal data (RCC-TOX LIMS). All other data were recorded on data sheets and compiled manually.

From the on-line recorded reproduction data, the following parameters were calculated: pre and post-implantation losses, embryonic and fetal deaths, live and dead fetuses, abnormal fetuses, fetal sex ratios and fetal body weights. For reproduction data, group mean values were calculated both on a litter basis and on a percentage per group basis. Mean fetal weights were calculated from the individual weights both on a per group and on a per litter basis.

3. Historical control data: Historical control data are provided for all reproductive and fetal findings observed in the current study. These historical control data were taken from 6 studies in the same strain of rat, evaluated by similar fetal examination criteria, in this laboratory in 2009 and 2010.

II. RESULTS

A. Maternal toxicity:

1. Mortality and Clinical Signs: All female animals survived until the scheduled necropsy. Test item-related clinical signs were observed in fourteen animals in the 30 mg/kg body weight/day dose group and considered adverse. Ataxia and decreased activity were noted in all animals with clinical signs, hunched posture (nos. 75, 76, 85, 91, 92, 96), prostrate (nos. 75, 90) and ruffled fur (nos. 76, 96) also were observed early in the treatment period. No clinical signs or observations were observed in ten animals in the 30 mg/kg body weight/day dose group (nos. 81, 82, 83, 84, 86, 87, 88, 89, 93, 94). At 7.5 and 15 mg/kg body weight/day and in the control group, no clinical signs or observations were noted during the study.

Table IIA1 Selected Maternal Clinical Signs[a]

```
                                Days
                                          1 1 1 1 1 1 1 1 1 1 2 2
                                0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1
-------------------------------------------------------------------------------
Group 1 (0 mg/kg)

No abnormality recorded.


Group 2 (7.5 mg/kg)

No abnormality recorded.


Group 3 (15 mg/kg)

No abnormality recorded.


Group 4 (30 mg/kg)

BEHAVIOR
- ATAXIA (3)                    G . . . . . 1 1 1 . . . 1 . 1 . 1 . . . . .
                                % . . . . . 4 1 1 . . . < . < . < . . . . .
- DECREASED ACTIVITY (3)        G . . . . . 1 1 1 . . . 1 . 1 . 1 . . . . .
                                % . . . . . 4 1 1 . . . < . < . < . . . . .
APPEARANCE
- PROSTRATE (1)                 G . . . . . 1 1 1 . . . . . . . . . . . . .
                                % . . . . . < < < . . . . . . . . . . . . .
- HUNCHED POSTURE (1)           G . . . . . 1 1 1 . . . 1 . 1 . . . . . . .
                                % . . . . . < < < . . . < . < . . . . . . .
- RUFFLED FUR (3)               G . . . . . 1 . . . . . . . . . 1 . . . . .
                                % . . . . . < . . . . . . . . . < . . . . .
No further abnormality recorded.
```

a Data taken from page 40 of the study report

2. <u>Body weight</u>: At 30 mg/kg body weight/day, body weights of the dams were statistically significantly reduced on day 8 post coitum onwards until termination, decreased from 3.5%-8.4% and increasing in magnitude with treatment duration. Body weight gain of the females was also statistically significantly reduced from day 7 post coitum until termination. Corrected body weight gain (corrected for the weight of the gravid uterus) was statistically significantly reduced when compared to the control group. The reduced body weights and corrected body weight gain in the 30 mg/kg body weight/day dose group were considered to be test article-related, however not considered adverse. At 7.5 and 15 mg/kg body weight/day, body weight, body weight gain and corrected body weight gain were not statistically significantly affected by treatment with the test article.

Table IIA2. Selected maternal body weight and body weight gain (mean±SD)[a]

		Group 1 0 mg/kg	Group 2 7.5 mg/kg	Group 3 15 mg/kg	Group 4 30 mg/kg
GESTATION	**PERIOD**				
Day 0	MEAN	200	201	199	202
	ST.DEV.	9	9	8	10
	N	24	23	21	23
Day 1	MEAN	204	206	206	207
	ST.DEV.	10	9	8	10
	N	24	23	21	23
Day 2	MEAN	209	211	210	212
	ST.DEV.	10	9	9	10
	N	24	23	21	23
Day 3	MEAN	213	215	214	216
	ST.DEV.	10	9	8	11
	N	24	23	21	23
Day 4	MEAN	218	219	219	220
	ST.DEV.	10	8	9	11
	N	24	23	21	23
Day 5	MEAN	221	221	222	223
	ST.DEV.	10	9	11	11
	N	24	23	21	23
Day 6	MEAN	223	224	225	226
	ST.DEV.	10	9	9	12
	N	24	23	21	23
Day 7	MEAN	226	227	228	222
	ST.DEV.	10	9	9	10
	N	24	23	21	23
Day 8	MEAN	230	230	231	222*
	ST.DEV.	11	10	9	9
	N	24	23	21	23
Day 9	MEAN	234	234	234	225*
	ST.DEV.	12	10	9	10
	N	24	23	21	23
Day 10	MEAN	238	238	240	228**
	ST.DEV.	11	10	10	12
	N	24	23	21	23

		Group 1 0 mg/kg	Group 2 7.5 mg/kg	Group 3 15 mg/kg	Group 4 30 mg/kg
GESTATION PERIOD					
Day 11	MEAN	244	244	245	233**
	ST.DEV.	11	11	9	12
	N	24	23	21	23
Day 12	MEAN	249	248	250	237**
	ST.DEV.	12	11	10	11
	N	24	23	21	23
Day 13	MEAN	252	252	254	241**
	ST.DEV.	12	11	10	11
	N	24	23	21	23
Day 14	MEAN	258	258	259	245**
	ST.DEV.	12	12	10	13
	N	24	23	21	23
Day 15	MEAN	265	264	265	250**
	ST.DEV.	13	13	10	12
	N	24	23	21	23
Day 16	MEAN	274	272	273	256**
	ST.DEV.	13	14	11	12
	N	24	23	21	23
Day 17	MEAN	283	282	283	265**
	ST.DEV.	15	16	12	15
	N	24	23	21	23
Day 18	MEAN	296	295	295	276**
	ST.DEV.	16	18	13	16
	N	24	23	21	23
Day 19	MEAN	308	305	305	285**
	ST.DEV.	17	19	14	17
	N	24	23	21	23
Day 20	MEAN	322	317	317	295**
	ST.DEV.	18	22	16	19
	N	24	23	21	23
Day 21	MEAN	337	333	333	309**
	ST.DEV.	19	25	17	19
	N	24	23	21	23

a Data obtained from pages 46-47 in the study report.
* Significantly different from control, $p<0.05$ (Dunnett-Test based on pooled variance)
** Significantly different from control, $p<0.01$ (Dunnett-Test based on pooled variance)
BW body weigh

3. <u>Food consumption</u>: At 30 mg/kg body weight/day, food consumption was statistically significantly reduced from day 6 post coitum onwards until termination, reductions from 19.4-33.3%. The mean reduction in food consumption from days 6-21 was 23.0%. This result was considered to be an effect of the treatment with the test item and adverse. Mean food consumption at 7.5 and 15 mg/kg body weight/day was not statistically significantly affected by treatment with the test article.

Table IIA 5.6.10/02-3. Food consumption (g/animal/day)[a]

		Group 1 0 mg/kg	Group 2 7.5 mg/kg	Group 3 15 mg/kg	Group 4 30 mg/kg
GESTATION PERIOD					
Days 0-3	MEAN	18.7	19.3	20.1**	19.7
	ST.DEV.	1.6	1.8	1.2	1.7
	N	24	23	21	23
Days 3-6	MEAN	20.6	20.7	21.3	20.6
	ST.DEV.	1.9	1.7	1.9	1.5
	N	24	23	21	23
MEAN OF MEANS Over GESTATION PERIOD (Days 0-6)		19.7	20.0	20.7	20.2

		Group 1 0 mg/kg	Group 2 7.5 mg/kg	Group 3 15 mg/kg	Group 4 30 mg/kg
GESTATION PERIOD					
Days 6-9	MEAN	21.0	20.6	20.2	14.0**
	ST.DEV.	2.1	1.7	1.8	1.7
	N	24	23	21	23
Days 9-12	MEAN	22.8	22.5	22.5	17.8**
	ST.DEV.	1.8	2.1	1.9	2.2
	N	24	23	21	23
Days 12-15	MEAN	24.4	24.1	24.5	19.5**
	ST.DEV.	2.0	2.4	1.6	2.1
	N	24	23	21	23
Days 15-18	MEAN	24.8	25.0	25.3	20.0**
	ST.DEV.	2.1	2.3	1.8	2.3
	N	24	23	21	23
Days 18-21	MEAN	24.6	24.0	23.9	19.4**
	ST.DEV.	1.7	2.0	1.8	2.8
	N	24	23	21	23
MEAN OF MEANS Over GESTATION PERIOD (Days 6-21)		23.5	23.3	23.3	18.1

a Data extracted from page 42-44 of the study report
* Statistically different from control, $p<0.05$ (Dunnett-Test based on pooled variance)
** Statistically different from control, $p<0.01$ (Dunnett-Test based on pooled variance)

4. Sacrifice and pathology:

Gross pathology: No macroscopical findings were noted in any dams at scheduled necropsy. No treatment-related external effects were noted in the fetuses.

5. Caesarean section data: At 30 mg/kg body weight/day, mean body weights of the fetuses on a litter basis were statistically significantly lower when compared to controls and considered to be related to the treatment with the test item. The live fetus weights

were decreased by10.0% at the high dose and considered adverse. There were no effects on mean fetal body weight at 7.5 or 15 mg/kg body weight/day. The sex ratios were not affected by treatment with the test item at any dose level, with statistical significance. However, live male pups decreased by 20% and live female pups increased by 18% at the highest dose relative to the control group. Sex ratios (% male/% female) were 56/44, 49/51, 53/47, and 46/54 at 0, 7.5, 15 and 30 mg/kg/day, respectively.

Table IIA 5.6.10/02-4. Cesarean section observations[a]

	GROUP 1 0 MG/KG	GROUP 2 7.5 MG/KG	GROUP 3 15 MG/KG	GROUP 4 30 MG/KG
NUMBER OF DAMS	24	23	21	23
CORPORA LUTEA	345	309	300	329
MEAN	14.4	13.4	14.3	14.3
ST.DEV.	2.3	1.7	1.6	1.6
PRE-IMPLANTATION LOSS	26	16	18	13
% OF CORP. LUTEA	7.5	5.2	6.0	4.0
MEAN	1.1	0.7	0.9	0.6
ST.DEV.	1.3	1.2	1.3	0.7
NUMBER OF DAMS AFFECTED	15	10	9	10
IMPLANTATION SITES	319	293	282	316
% OF CORP. LUTEA	92.5	94.8	94.0	96.0
MEAN	13.3	12.7	13.4	13.7
ST.DEV.	2.5	2.3	1.6	1.7
POST-IMPLANTATION LOSS	24	20	27	32
% OF IMPL. SITES	7.5	6.8	9.6	10.1
MEAN	1.0	0.9	1.3	1.4
ST.DEV.	1.7	1.3	1.5	1.3
NUMBER OF DAMS AFFECTED	13	12	12	18
IMPLANTATION SITE SCARS	0	0	0	0
EMBRYONIC/FETAL DEATHS TOTAL	24	20	27	32
EMBRYONIC RESORPTIONS	24	19	26	29
% OF IMPL. SITES	7.5	6.5	9.2	9.2
MEAN	1.0	0.8	1.2	1.3
ST.DEV.	1.7	1.3	1.5	1.2
NUMBER OF DAMS AFFECTED	13	11	11	17
FETAL RESORPTIONS	0	1	1	3
% OF IMPL. SITES		0.3	0.4	0.9
MEAN		0.0	0.0	0.1
ST.DEV.		0.2	0.2	0.3
NUMBER OF DAMS AFFECTED		1	1	3
FETUSES				
TOTAL FETUSES	295	273	255	284
% OF IMPL. SITES	92.5	93.2	90.4	89.9
MEAN	12.3	11.9	12.1	12.3
ST.DEV.	2.0	3.0	2.1	1.5
LIVE FETUSES	295	273	255	284
DEAD FETUSES	0	0	0	0
FETUSES WITH EXTERNAL ABNORMALITY	1	0	0	0
% OF FETUSES	0.3			
MEAN	0.0			
ST.DEV.	0.2			
NUMBER OF DAMS AFFECTED	1			
LIVE FETUSES WITH ABNORMALITIES AT EXTERNAL EXAMINATION	1	0	0	0
DEAD FETUSES WITH ABNORMALITIES AT EXTERNAL EXAMINATION	0	0	0	0

	GROUP 1 0 MG/KG	GROUP 2 7.5 MG/KG	GROUP 3 15 MG/KG	GROUP 4 30 MG/KG
NUMBER OF DAMS	24	23	21	23
SEX OF FETUSES				
TOTAL MALES	165	134	135	132
% OF FETUSES	55.9	49.1	52.9	46.5
MEAN	6.9	5.8	6.4	5.7
ST.DEV.	2.6	2.4	2.3	1.8
TOTAL FEMALES	130	139	120	152
% OF FETUSES	44.1	50.9	47.1	53.5
MEAN	5.4	6.0	5.7	6.6
ST.DEV.	2.0	2.7	2.0	1.9
LIVE MALES	165	134	135	132
LIVE FEMALES	130	139	120	152
WEIGHTS OF LIVE FETUSES (G) (LITTER BASIS)				
TOTAL FETUSES				
N (LITTERS)	24	23	21	23
MEAN	4.8	4.8	4.7	4.3 **
ST.DEV.	0.3	0.3	0.3	0.3
MALES				
N (LITTERS)	24	23	21	23
MEAN	5.0	4.9	4.8	4.5 **
ST.DEV.	0.3	0.3	0.3	0.3
FEMALES				
N (LITTERS)	24	23	21	23
MEAN	4.7	4.7	4.6	4.2 **
ST.DEV.	0.3	0.3	0.3	0.3

a Data obtained from pages 55-56 in the study report.
* Significantly different from control, p<0.05, ** Significantly different from control, p<0.01 (Dunnett-Test based on pooled variance)

B. <u>Developmental Toxicity</u>:

1. <u>External examination</u>: No treatment-related effects were noted. At scheduled caesarean section, one fetus in the control group (no. 861, litter no. 17) had thread-like tail and absent anus.

2. <u>Visceral examination</u>: Abnormalities were seen in five fetuses in five litters at 30 mg/kg body weight/day and were considered adverse. Two fetuses from two litters had multiple malformations: fetus 726 small pituitary, great vessel abnormalities and inter-ventricular septal defect; fetus 852 great vessel abnormalities and inter-ventricular septal defect of the heart. In the three additional fetuses single findings in one fetus per finding of situs inversus total, severely thin diaphragm and severely dilated renal pelvis and ureter were noted. As these were isolated occurrences and known findings in this strain of rat in this laboratory they were considered to be incidental and not related to the treatment with the test item.

Based on both fetal and litter values, the incidence of thymus long cranial was higher in the 15 and 30 mg/kg body weight/day groups than in controls (controls had 60% incidence in the litter and 14% in the fetuses). Statistical significances were attained at 15 mg/kg body weight/day on fetal basis and at 30 mg/kg body weight/day dose group both

on fetal and litter basis. At 7.5 mg/kg body weight/day, the incidence of thymus long cranial was not statistically different from the control group and was within the historical background range. At 15 mg/kg body weight/day, the fetal value was marginally higher than the historical background range and the litter value was within the historical range. At 30 mg/kg body weight/day, the values were clearly higher than the historical control range. In the absence of increased incidences of findings in other related structures, however, the higher incidence of an isolated minor variation which is commonly seen in this laboratory, was considered to be of no biological significance in terms of fetal development. Other variations observed at all dose levels were isolated incidences or were within the historical control range.

SYN545192: Rat Developmental Toxicity

- There was an increase in the litter incidence of "thymus long cranial" at 30 mg/kg bw/day that was outside of the lab's historical control range

Dose (mg/kg/day)		0		7.5		15		30	
No. fetuses examined		154		140		134		147	
No. of litter examined		24		23		21		23	
		n	%	n	%	n	%	n	%
Thymus long cranial	Fetus	11	7	16	11	24**	18	35**	24
	Litter	7	29	10	43	11	52	17**	74

Fisher's Exact Test Significant at 5%(*) or 1%(**) level. Lab's historical range: up to 60% litter incidence; up to 14% fetal incidence

- Considered not to be an adverse finding
  - Frequently seen in control animals in the test laboratory
  - Part of the normal spectrum of physiological change as the thymic tissue migrates to the thoracic inlet
  - Not associated with any functional abnormalities

Table IIA 5.6.10/02-6. Visceral Examinations[a]

		Group 1 0 mg/kg		Group 2 7.5 mg/kg		Group 3 15 mg/kg		Group 4 30 mg/kg	
Number of fetuses examined		154		141		134		147	
Number of litters examined		24		23		21		23	
Incidences of fetuses/litters with		N	%	N	%	N	%	N	%
Abnormalities									
Thoracic and abdominal situs inversus	Fetus Litter	0		0		0		1 1	1 4
Pituitary small. Origins of ascending aorta and pulmonary trunk malpositioned and heart interventricular septal defect	Fetus Litter	0		0		0		1 1	1 4
Aortic arch narrow, origins of ascending aorta and pulmonary trunk malpositioned and heart interventricular septal defect. Lung two lobes only. Azygos vein persisting into abdomen. Abdominal situs inversus and liver lobes misshapen/absent	Fetus Litter	0		0		0		1 1	1 4
Diaphragm severely thin localized	Fetus Litter	0		0		0		1 1	1 4
Renal pelvis and ureter severely dilated	Fetus Litter	0		0		0		1 1	1 4
Possible artefact °									
Head cranium ruptured and brain herniated	Fetus Litter	0		0		1 1	1 5	0	
Variations									
Brain/spinal cord perimeningeal haemorrhage	Fetus Litter	7 7	5 29	6 5	4 22	6 6	4 29	11 8	7 35
Eye retinal folds	Fetus Litter	1 1	1 4	0		0		0	
Eye lens cut surface abnormal texture	Fetus Litter	0		1 1	1 4	0		0	
Eye vitreous chamber haemorrhage	Fetus Litter	0		0		2 2	1 10	0	
Thyroid large	Fetus Litter	0		2 1	1 4	0		0	
Thyroid small	Fetus Litter	0		1 1	1 4	0		0	
Thymus long cranial	Fetus Litter	11 7	7 29	16 10	11 43	24## 11	18 52	35## 17##	24 74
Subclavian artery origin malpositioned	Fetus Litter	1 1	1 4	0		1 1	1 5	1 1	1 4
Azygos vein bilateral	Fetus Litter	0		0		0		1 1	1 4

		Group 1 0 mg/kg		Group 2 7.5 mg/kg		Group 3 15 mg/kg		Group 4 30 mg/kg	
Number of fetuses examined		154		141		134		147	
Number of litters examined		24		23		21		23	
Incidences of fetuses/litters with		N	%	N	%	N	%	N	%
Variations continued									
Diaphragm tendinous region thin localized	Fetus Litter	10 9	6 38	7 6	5 26	7 6	5 29	8 6	5 26
Abdomen internal haemorrhage	Fetus Litter	0		0		0		1 1	1 4
Liver abnormal lobation	Fetus Litter	14 11	9 46	12 10	9 43	5 5	4 24	17 11	12 48
Renal pelvis dilated	Fetus Litter	0		0		3 2	2 10	4 4#	3 17
Ureter dilated	Fetus Litter	0		1 1	1 4	0		1 1	1 4
Testis malpositioned	Fetus Litter	3 3	2 13	1 1	1 4	6 6	4 29	3 3	2 13
Umbilical artery left-sided	Fetus Litter	17 13	11 54	19 11	13 48	12 8	9 38	13 11	9 48
Subcutaneous haemorrhage	Fetus Litter	7 4	5 17	1 1	1 4	4 3	3 14	5 4	3 17
Fetuses with abnormality		**0**		**0**		**0**		**5#**	**3**
Litters with abnormality		**0**		**0**		**0**		**5#**	**22**
Fetuses with any finding		60	39	53	38	62	46	78#	53
Litters with any finding		24	100	21	91	20	95	23	100

a Data extracted from pages 65-66 of the study report.
/ ## : Fisher's Exact Test significant at level 5% (#) or 1% (##)
◦ Excluded from number of fetuses and litters with abnormality

3. <u>Skeletal examinations</u>: The following cartilage abnormalities occurred in one fetus in one litter: costal cartilage 1 short and costal cartilage 2 branched at 7.5 mg/kg body weight/day, and in another fetus costal cartilages 1-2 were fused at 30 mg/kg body weight/day. These findings were considered to be incidental. Bone or cartilage abnormalities (multiple abnormalities, cervical and thoracic scoliosis, cervical vertebral bodies split or absent caudal vertebrae) were seen in 4 fetuses in 4 litters in the control group.

Based on litter evaluation, the following findings occurred in statistically significantly increased incidences: Non-ossified cervical vertebral body 2 and incompletely ossified sternebra 5 at 30 mg/kg body weight/day. Based on fetal evaluation, the following findings occurred in statistically significantly increased incidences: Non-ossified cervical vertebral bodies 1 and 2, incompletely ossified sternebra 5, non-ossified proximal phalanges of digits 5 and non-ossified calcaneus at 30 mg/kg body weight/day. These findings were outside of the historical background range and indicated a slight delay in ossification at 30 mg/kg body weight/day which correlated with the lower fetal body weight in this dose group and were considered to be adverse. All other incidences were

similar in the other test item treated groups and the control group and/or were within the range of historical reference data.

No test item-related findings were observed during cartilage examination. The incidence of branched distal extremity of costal cartilage 8 on the right was statistically significantly increased at 30 mg/kg body weight/day when evaluated on individual basis only but since there was no dose dependent increase at litter evaluation this finding was considered to be incidental. All other cartilageous variations were similar in the test item treated groups and the control group and / or were within the range of historical reference data.

Table IIA 5.6.10/02-7. Skeletal Examinations[a]

		Group 1 0 mg/kg		Group 2 7.5 mg/kg		Group 3 15 mg/kg		Group 4 30 mg/kg	
Number of fetuses examined		141		132		121		137	
Number of litters examined		24		23		21		23	
Incidences of fetuses/litters with		N	%	N	%	N	%	N	%
Bone and cartilage abnormalities									
Cervical scoliosis and thoracic scoliosis	Fetus	1	1	0		0		0	
	Litter	1	4						
Cervical vertebral body split	Fetus	2	1	0		0		0	
	Litter	2	8						
Costal cartilage of vertebrosternal rib fused / short / branched	Fetus	0		1	1	0		1	1
	Litter			1	4			1	4
All caudal vertebrae absent (*tail threadlike at external examination)*	Fetus	1	1	0		0		0	
	Litter	1	4						
Bone variations									
Skull zygomatic arch fusion	Fetus	1	1	2	2	2	2	3	2
	Litter	1	4	2	9	2	10	2	9
Cervical rib	Fetus	2	1	0		0		0	
	Litter	2	8						
Pectoral girdle scapula slightly misshapen	Fetus	2	1	0		0		2	1
	Litter	2	8					2	9
Thoracic vertebral body dumbbell ossification or bipartite ossification	Fetus	0		1	1	1	1	0	
	Litter			1	4	1	5		
Sternebra short unilateral and isolated ossification site adjacent	Fetus	0		0		1	1	0	
	Litter					1	5		
Sternebra offset ossification sites	Fetus	5	4	3	2	2	2	2	1
	Litter	5	21	1	4	2	10	2	9
Sternebra bipartite ossification	Fetus	3	2	0		1	1	0	
	Litter	3	13			1	5		
Pelvic girdle malpositioned caudal									
Total	Fetus	5	4	12	9	6	5	7	5
	Litter	3	13	4	17	4	19	6	26
Unilateral	Fetus	4	3	3	2	2	2	4	3
	Litter	2	8	3	13	2	10	4	17
Bilateral	Fetus	1	1	9##	7	4	3	3	2
	Litter	1	4	4	17	4	19	2	9

		Group 1 0 mg/kg		Group 2 7.5 mg/kg		Group 3 15 mg/kg		Group 4 30 mg/kg	
Number of fetuses examined		**141**		**132**		**121**		**137**	
Number of litters examined		**24**		**23**		**21**		**23**	
Incidences of fetuses/litters with		**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Cartilage variations									
Cervical or thoracic vertebral body dumbbell-shaped	Fetus Litter	0		0		1 1	1 5	1 1	1 4
Costal cartilages asymmetrically aligned at sternum	Fetus Litter	4 4	3 17	3 1	2 4	2 2	2 10	2 2	1 9
Costal cartilage of false rib branched	Fetus Litter	1 1	1 4	0		0		0	
Fetuses with abnormality		**4**	**3**	**1**	**1**	**0**		**1**	**1**
Litters with abnormality		**4**	**17**	**1**	**4**			**1**	**4**
Fetuses with any finding		16	11	17	13	11	9	14	10
Litters with any finding		11	46	6	26	8	38	12	52

a Data extracted from pages 67-68 of the study report.
/ ## : Fisher's Exact Test significant at level 5% (#) or 1% (##)

III. Discussion

A. <u>Investigator's Conclusions (extracted from page 35 in the study report)</u> –

The dose level of 30 mg/kg body weight/day produced obvious maternal toxicity. Clinical signs of decreased activity, hunched posture, ataxia, prostrate and ruffled fur were noted during the treatment period. Food consumption, body weight, body weight gain and corrected body weight gains were statistically significantly reduced. Fetal weights were also statistically significantly reduced in this dose group and at skeletal examination a slight delay in ossification was observed. No test item-related findings were found during the study in the 7.5 and 15 mg/kg body weight/ day dose groups. Based on the result of this study, the NOEL (No-Observed-Effect-Level) for maternal effects was 15 mg/kg body weight/day. The NOEL for developmental toxicity was 15 mg/kg body weight/day based on effects on fetal weight and ossification at 30 mg/kg body weight/day. No biologically significant morphological alterations, including teratogenicity, were observed in this study.

B. <u>Reviewer's Conclusions</u> – The reviewer is in agreement with the investigator on the selection of the NOAEL and LOAEL values. However, the Agency does not consider the body weight decreases less than 10% in the dams to be adverse, nor the accompanying body weight gain decreases. The single finding of thymus long cranial at 15 mg/kg/day in the embryos does exceed the historical control range, however, the multiple effects at 30 mg/kg/day are more consistently adverse by the weight of evidence. The Agency does not consider the thymus long cranial finding to be adverse in the absence of corroborating evidence. The immunotoxicity study in mice (MRID 48604461) did not indicate dose-responsive nor statistically significant effects on thymus weights relative to body weight at doses up to 97.1 mg/kg/day.

C. <u>Deficiencies</u> - Multiple deviations from the study protocol were documented,

however, these alterations were not determined to substantially alter the conclusions of the study.

D. <u>References</u> –

1. Harlan Laboratories Study C73668: SYN545192 - Dose Range-Finding Prenatal Developmental Study in the Han Wistar Rat

2. E. Salewski: Arch. Exp. Path. Pharmak. 247, pp. 367-368 (1964)

3. Modification of M.V. Barrow and W.J. Taylor: A rapid method for detecting malformations in rat fetuses. J. Morph. 127, pp. 291- 306 (1969)

4. Modification of M. Inouye: Differential staining of cartilage and bone in fetal mouse skeleton by Alcian blue and Alizarin red-S. Congenital Anomalies 16, pp. 171-173 (1976)

5. C.W. Dunnett: A Multiple Comparison Procedure for Comparing Several Treatments with a Control, J. Amer. Statist. Assoc. 50, pp. 1096-1121 (1955).

6. R.A. Fisher: Statistical Methods for Research Workers, Oliver and Boyd, Edinburgh (1950).

7. Harlan Laboratories Study C80036: SYN545192 - Transfer and Validation of an Analytical Method for the Determination of SYN545192 in 0.5% (w/v) Carboxymethylcellulose (CMC) Aqueous Suspension

Report:	IIA 5.6.11/02 Sawhney Coder P (2011). **SYN545192 – A Prenatal Developmental Toxicity Study in New Zealand White Rabbits**WIL Research Laboratories, LLC, Ashland, OH. Laboratory Report No. WIL-639054. Issue date: August 26, 2011. Unpublished. MRID #48604453

Guidelines: Prenatal Developmental Study (rabbit) OECD 414 (2001): OPPTS 870.3700 (1998): 2004/73/EC B.31 (2004): MAFF, 12 Nousan No. 8147 (2000)

Sponsor: Syngenta Crop Protection, LLC 410 Swing Road Post Office Box 18300 Greensboro, NC 27419-8300 USA

Executive Summary: The test substance, SYN545192, in the vehicle, 0.5% (w/v) aqueous medium viscosity carboxymethylcellulose (CMC), was administered orally by gavage to three groups of 25 time-mated female New Zealand White [Hra:(NZW)SPF] rabbits once daily from gestation days 7 through 28. Dose levels were 10, 20, and 35 mg/kg/day administered at a dose volume of 10 mL/kg. A concurrent control group composed of 25 time-mated females received the vehicle (0.5% medium viscosity CMC) on a comparable regimen. The females were approximately 6 months of age at the initiation of dose administration. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. On gestation day 29, a laparohysterectomy was performed on each female. The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. In addition, the livers from all females examined at the scheduled necropsy were weighed. The fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations.

All females survived to the scheduled necropsy on gestation day 29. There were no test substance-related clinical or macroscopic findings for females at any dose level with statistical significance and/or dose response. There were no test substance-related effects on mean maternal body weights, net body weights, net body weight losses, gravid uterine weights, or food consumption at any dose level with statistical significance and/or dose response. Marginal effects on maternal body weight gain at 20 and 35 mg/kg/day during gestation days 13-20 were considered to be non-adverse. Mean maternal absolute liver weights and liver weights adjusted for net body weight were unaffected by test substance administration at all dose levels. Intrauterine growth and survival and fetal morphology were unaffected by test substance administration at all dose levels.

Based on the lack of maternal and embryo/fetal developmental toxicity with statistical significance and/or dose responsiveness, **the NOAEL (no-observed adverse- effect level) for both maternal toxicity and embryo/fetal developmental toxicity was 35 mg/kg/day** (the highest dose level evaluated) when SYN545192 was administered orally by gavage to pregnant New Zealand White rabbits. **A study LOAEL (lowest-observed adverse- effect level) was not established.** However, the rabbit developmental range-

finding study did demonstrate adverse effects at 50 mg/kg/day (decreased maternal body weight gain was observed throughout the majority of gestation, 2 of 10 animals were euthanized due to excessive weight loss, and a third animal aborted late in gestation).

This study is **acceptable/guideline** and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3700; OECD 414) in rats.

Compliance: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No claim of CBI was made for any information contained in this document.

I. MATERIALS AND METHODS

A. Materials:

Test Material:	SYN545192
Description:	Coarse, light yellow powder
Lot/Batch number:	SMU9BP005
Purity:	97.0%
Stability of test compound:	28 February 2013 expiration date
Vehicle:	0.5% (w/v) aqueous high viscosity carboxymethylcellulose
Positive control:	None

Test Animals:

Species	Rabbit
Strain	New Zealand White [Hra:(NZW)SPF]
Age at dosing	Approximately 5.5 months
Weight at dosing	2900 g to 4500 g
Source	Covance Research Products, Inc., Kalamazoo, MI
Housing	Individually in suspended, stainless steel cages
Acclimatization period	Not reported
Diet	study, PMI Nutrition International, LLC, Certified Rabbit LabDiet® 5322, in gradual 25 g increments (3x/day) starting the day of receipt, and *ad libitum* thereafter, supplemented with kale if food consumption dropped below 10g/day for 3 consecutive days
Water	Reverse osmosis-purified (on-site) drinking water *ad libitum*
Environmental conditions	Temperature: 19 +/- 3°C Humidity: 50 +/- 20% Air changes: 10 fresh air changes per hour Photoperiod: 12 hour light/12 hour dark

B. Study Design and Methods:

1. In-life dates: Start: 20 October 2009. (first gestation day 0)
End: 20 January 2010 (last fetal skeletal examination)

2. Mating procedure: Time-mated female New Zealand White [Hra:(NZW)SPF] rabbits from Covance Research Products, Inc., Kalamazoo, MI, were used as the test system on this study. The time mated rabbits were received on gestation day 1, 2, or 3; a breeding record was provided by the supplier and is maintained in the study records. The animals were approximately 5.5 months old upon receipt. Each female was examined by a qualified technician on the day of receipt. Body weights were recorded on gestation day 4 and food consumption was recorded during gestation days 4-7. Each rabbit was uniquely identified by a color-coded plastic ear tag displaying the animal number. The rabbits were observed twice daily for mortality and general changes in appearance and behavior. Body weights were recorded by the supplier on gestation day 0 under non-GLP conditions.

3. Animal assignment and treatment: Animals were randomly assigned to test groups based on body weight stratification in a block design using the WIL Toxicology Data Management System (WTDMS^{TM}) as shown in Table IIA 5.6.11/02-1.

Table IIA 5.6.11/02–1: Animal Assignment

Dose (mg/kg bw/day)	0	10	20	35
# Females	25	25	25	25

4. Dose selection rationale: Dose levels were selected based on the results of previous 10-day tolerability (Sawhney Coder, 2011; WIL-639038 and Sawhney Coder, 2011; WIL-639041) and dose range-finding prenatal developmental toxicity studies (Sawhney Coder, 2011, WIL-639039). In the 10-day tolerability studies, dose levels of 150 mg/kg/day and above were not well tolerated, as evidenced by mortality, moribundity, reduced body weights, and/or decreased food consumption; dose levels of 15 and 50 mg/kg/day were well tolerated. In the dose range-finding prenatal developmental toxicity study, dose levels of 50 and 100 mg/kg/day resulted in maternal moribundity, abortion, reduced body weights, and decreased food consumption; intrauterine growth and survival were unaffected at dose levels of 25 and 50 mg/kg/day. However, in the dose range-finding study in pregnant rabbits (Sawhney Coder, 2011, WIL-639039), decreased maternal body weight gain was observed throughout the majority of gestation at a dose of 50 mg/kg/day, and 2 of 10 animals (20%) were euthanized due to excessive weight loss, and a third animal aborted late in gestation. Based on these results, dose levels of 10, 20, and 35 mg/kg/day were selected for the current study. The selected route of administration for this study was oral (gavage) because this is a potential route of human exposure.

5. Dosage preparation and analysis: The CMC vehicle solution was prepared approximately every 4 days for administration to the control group (Group 1) and for preparation of the test substance suspensions; aliquots were prepared for daily dispensation to the control group and stored refrigerated (approximately 2°C to 8ºC). The vehicle was mixed throughout preparation, sampling, and dose administration procedures.

Dosing suspensions were prepared at the test substance concentrations indicated in the following table, with Group 1 being the control group at 0 mg/kg/day dosing:

Group Number	Test Substance	Dose Level (mg/kg/day)	Test Substance Concentration (mg/mL)
2	SYN545192	10	1
3	SYN545192	20	2
4	SYN545192	35	3.5

The test substance suspensions were prepared approximately every 4 days as single suspensions for each dose level, divided into aliquots for daily dispensation, and stored refrigerated (approximately 4ºC), with the following exceptions. The homogeneity and concentration of suspensions prepared on 26 October 2009 were found to be outside acceptable specifications (concentrations 85% to 115% of target with RSD of ≤10%), these suspensions were administered to animals on the first day of dosing (27 October 2009). Due to a 3-day breeding stagger, only one-third of the rabbits on study received this preparation. A fresh batch of dosing suspensions that was within specifications was prepared on 27 October 2009 for administration to the animals beginning the following day. The test substance suspensions were stirred continuously throughout the preparation, sampling, and dose administration procedures. The pH measurements of the first dosing suspensions were 7.46, 7.09, 7.07, and 7.09 for the control, 1, 2, and 3.5 mg/mL suspensions, respectively. The first dosing suspensions were visually inspected by the Study Director and were found to be visibly homogeneous and acceptable for administration.

Results:

Homogeneity Analysis: 1.1-15.0% relative standard deviation from mean

Stability Analysis: homogeneous, and were stable when refrigerated (approximately 4ºC) for up to 5 days or frozen (approximately -70ºC) for up to 22 days.

Concentration Analysis: 82.2-108.0% of target

However, the suspensions prepared on 26 October 2009 at concentrations of 1, 2, and 3.5 mg/mL did not meet the WIL Research SOP requirements for concentration or homogeneity.

6. Dosage administration: The vehicle and test substance suspensions were administered orally by gavage, via 22-French rubber catheters (C.R. Bard, Inc., Covington, GA), once daily during gestation days 7 through 28. The dose volume for all groups was 10 mL/kg. Following administration of each dose, the catheter was flushed with 5 mL of deionized water to ensure delivery of the entire dose. Individual doses were based on the most recently recorded body weights to provide the correct mg/kg/day dose. All animals were dosed at approximately the same time each day.

C. Observations:

1. Maternal observations and evaluations: All rabbits were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality. Individual clinical observations were recorded from the day of receipt through gestation day 29 (prior to dose administration during the treatment period). Animals were also observed for signs of toxicity approximately 4 hours following dose administration. The absence or presence of findings was recorded for individual animals.

Individual maternal body weights were recorded on gestation days 0 (by the supplier), 4, and 7-29 (daily). Group mean body weights were calculated for each of these days. Mean body weight changes were calculated by cumulatively normalizing daily body weight change from the first day of dose administration (gestation day 7), and for gestation days 0-4, 0-7, 7-10, 10-13, 13-21, and 21-29. Gravid uterine weight was collected and net body weight (the gestation day 29 body weight exclusive of the weight of the uterus and contents) and net body weight change (the gestation day 0 29 body weight change exclusive of the weight of the uterus and contents) were calculated and presented for each gravid female at the scheduled laparohysterectomy.

The laparohysterectomies and macroscopic examinations were performed blind to treatment group. All rabbits were euthanized on gestation day 29 by an intravenous injection of sodium pentobarbital via the marginal ear vein. The thoracic, abdominal, and pelvic cavities were opened by a ventral mid-line incision, and the contents were examined. In all instances, the *postmortem* findings were correlated with the ante mortem comments, and any abnormalities were recorded. The uterus and ovaries were then exposed and excised. The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed and opened, and the number and location of all fetuses, early and late resorptions, and the total number of implantation sites were recorded. The placentae were also examined. The individual uterine distribution of implantation sites was documented using the following procedure. All implantation sites, including resorptions, were numbered in consecutive order beginning with the left distal to the left proximal uterine horn, noting the position of the cervix, and continuing from the right proximal to the right distal uterine horn. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss (Salewski, 1964). Maternal tissues with gross lesions were preserved in 10% neutral-buffered formalin for possible future histopathologic examination. Representative sections of corresponding organs from a sufficient number of control animals were retained for comparison. The liver of each female was weighed and placed in 10% neutral-buffered formalin. Liver to net body weight ratios were calculated (liver weight x 100/net body weight). The carcass of each female was then discarded.

2. Fetal observations: Fetal examinations were performed blind to treatment group. Each viable fetus was examined externally, individually weighed, euthanized by hypothermia followed by an intra-thoracic injection of sodium pentobarbital (if necessary), and tagged for identification. Findings for the nonviable fetus are included on

the fetal table or in Appendix 4. The detailed external examination of each fetus included, but was not limited to, an examination of the eyes, palate, and external orifices, and each finding was recorded. Non-viable fetuses (if the degree of autolysis was minimal or absent) were examined, the crown-rump length measured, weighed, sexed, and tagged individually. Crown-rump measurements, degrees of autolysis, and gross examinations, if possible, were recorded for late resorptions, and the tissues were discarded.

Each viable fetus was subjected to a visceral examination using a modification of the Stuckhardt and Poppe fresh dissection technique to include the heart and major blood vessels (Stuckhardt and Poppe, 1984). The sex of each fetus was determined by internal examination. Fetal kidneys were examined and graded for renal papillae development (Woo and Hoar, 1972). Heads from all fetuses were examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol. Following fixation in alcohol, each fetus was stained with Alizarin Red S (Dawson, 1926) and Alcian Blue (Inouye, 1976). External, visceral, and skeletal findings were recorded as developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity and/or occur at a high incidence, representing slight deviations from normal) or malformations (those structural anomalies that alter general body conformity, disrupt or interfere with normal body function, or may be incompatible with life).

D. Data Analysis

1. Statistical analyses: All statistical tests were performed using appropriate computing devices or programs. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test substance-treated group to the control group. Each mean was presented with the standard deviation (S.D.), standard error (S.E.), and the number of animals (N) used to calculate the mean. Data from nongravid females were excluded from statistical analyses. Due to the use of significant figures and the different rounding conventions inherent in the types of software used, the means and standard deviations on the summary and individual tables may differ slightly. Where applicable, the litter was used as the experimental unit. Mean maternal body weights (absolute and net), body weight changes (absolute and net) and food consumption, gravid uterine weights, absolute liver weights, numbers of corpora lutea, implantation sites, viable fetuses, and mean fetal body weights (separately by sex and combined) were subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) followed by Dunnett's test (Dunnett, 1964). Maternal performance data (*e.g.*, proportion of females with live fetuses at termination, abortions, and total resorptions) and macroscopic findings were analyzed by Fisher's Exact Test (Steel and Torrie, 1980), comparing each test substance-treated group to the control group. The incidence of fetal malformations and developmental variations (external, visceral, and skeletal) were summarized as the proportion of fetuses affected and the proportion of litters affected. The proportions were analyzed by a two-tailed Fisher's Exact Test (Steel and Torrie, 1980), comparing each test substance-treated group to the control group.

Statistical analyses of mean liver weights (using net body weight as the covariate) and mean litter proportions of the prenatal data were performed by BioSTAT Consultants, Inc., Portage, MI, using the SAS System (SAS Institute, Inc., 2002 2003) Software (Appendix 5). Mean liver weights were subjected to a parametric one-way analysis of covariance (ANCOVA) (SAS Institute, Inc., 2002-2003), with net body weight as the covariate, followed by Dunnett's test, to determine intergroup differences. No statistical analysis was performed for liver to net body weight ratios as the analysis of covariance provides a better method of allowing for differences in final body weight (Shirley, 1977). Mean litter proportions (percent per litter) of prenatal data (viable and nonviable fetuses, early and late resorptions, total resorptions, pre- and postimplantation loss, and fetal sex distribution), were summarized as the group proportion affected. The litter based mean percentage was summarized and subjected to a double arcsine transformation (Freeman and Tukey, 1950) followed by ANOVA and Dunnett's test.

2. Indices: Intrauterine data were summarized using two methods of calculation. An example of each method of calculation follows:

Group Mean Litter Basis:

$$\text{Postimplantation Loss/Litter} = \frac{\text{No. Dead Fetuses, Resorptions (Early/Late)/Group}}{\text{No. Gravid Females/Group}}$$

Proportional Litter Basis:

$$\text{Summation Per Group (\%)} = \frac{\text{Sum of Postimplantation Loss/Litter (\%)}}{\text{No. Litters/Group}}$$

Where:

$$\text{Postimplantation Loss/Litter (\%)} = \frac{\text{No. Dead Fetuses, Resorptions (Early/Late)/Litter}}{\text{No. Implantation Sites/Litter}} \times 100$$

The fetal developmental findings were summarized by: 1) presenting the incidence of a given finding both as the number of fetuses and the number of litters available for examination in the group; and 2) considering the litter as the basic unit for comparison and calculating the number of affected fetuses in a litter on a proportional basis as follows:

$$\text{Summation per Group (\%)} = \frac{\text{Sum of Viable Fetuses Affected/Litter (\%)}}{\text{No. Litters/Group}}$$

Where:

$$\text{Viable Fetuses Affected/Litter (\%)} = \frac{\text{No. Viable Fetuses Affected/Litter}}{\text{No. Viable Fetuses/Litter}} \times 100$$

3. Historical control data: Historical control data were provided for all reproductive and fetal findings. The current WIL historical control database (Version 1.3) is composed of 1280 dams from 59 datasets. Additionally, 10,254 fetuses from 1201 litters received external, visceral and skeletal examinations.

II. RESULTS

A. Maternal toxicity:

1. Mortality and Clinical signs: All females survived to the scheduled necropsy on gestation day 29. No test substance-related clinical findings were noted at the daily examinations or approximately 4 hours following dose administration at any dose level. Findings noted in the test substance-treated groups, including decreased defecation and soft stool, occurred infrequently, at similar frequencies in the control group, and/or in a manner that was not dose-related.

Table IIA 5.6.11/02–1: Mortality and Clinical Signs[a]

DOSE GROUP :	1		2		3		4	
	NO.	%	NO.	%	NO.	%	NO.	%
FEMALES ON STUDY	25		25		25		25	
FEMALES THAT ABORTED OR DELIVERED	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES THAT DIED	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES THAT ABORTED	0	0.0	0	0.0	0	0.0	0	0.0
NONGRAVID	0	0.0	0	0.0	0	0.0	0	0.0
GRAVID	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES THAT WERE EUTHANIZED	0	0.0	0	0.0	0	0.0	0	0.0
NONGRAVID	0	0.0	0	0.0	0	0.0	0	0.0
GRAVID	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES EXAMINED AT SCHEDULED NECROPSY	25	100.0	25	100.0	25	100.0	25	100.0
NONGRAVID	1	4.0	2	8.0	1	4.0	0	0.0
GRAVID	24	96.0	23	92.0	24	96.0	25	100.0
WITH RESORPTIONS ONLY	0	0.0	0	0.0	0	0.0	0	0.0
WITH VIABLE FETUSES	24	100.0	23	100.0	24	100.0	25	100.0
TOTAL FEMALES GRAVID	24	96.0	23	92.0	24	96.0	25	100.0

1- 0 MG/KG/DAY 2- 10 MG/KG/DAY 3- 20 MG/KG/DAY 4- 35 MG/KG/DAY
FEMALES THAT ABORTED OR DELIVERED AND FEMALES THAT WERE EXAMINED AT THE SCHEDULED NECROPSY WITH RESORPTIONS ONLY OR WITH VIABLE FETUSES WERE ANALYZED STATISTICALLY.
None significantly different from control group using 2-tailed Fisher's Exact test

----- F E M A L E -----

TABLE RANGE: 10-23-09 TO 11-20-09 GROUP:	1	2	3	4
NORMAL				
-NO SIGNIFICANT CLINICAL OBSERVATIONS	611/25	548/25	517/25	550/25
DISPOSITION				
-SCHEDULED EUTHANASIA; GESTATION DAY 29	25/25	25/25	25/25	25/25
BODY/INTEGUMENT				
-HAIR LOSS UROGENITAL AREA	2/ 1	0/ 0	8/ 1	0/ 0
-WET RED MATERIAL UROGENITAL AREA	0/ 0	1/ 1	0/ 0	0/ 0
-HAIR LOSS VENTRAL ABDOMINAL AREA	0/ 0	0/ 0	6/ 1	0/ 0
-HAIR LOSS RIGHT HINDLIMB	0/ 0	0/ 0	2/ 1	0/ 0
-HAIR LOSS LEFT HINDLIMB	0/ 0	0/ 0	1/ 1	1/ 1
-WET RED MATERIAL BASE OF TAIL	0/ 0	1/ 1	0/ 0	0/ 0
-WET RED MATERIAL RIGHT HINDLIMB	0/ 0	1/ 1	0/ 0	0/ 0
-WET RED MATERIAL LEFT HINDLIMB	0/ 0	1/ 1	0/ 0	0/ 0
CARDIO-PULMONARY				
-RALES	2/ 1	0/ 0	1/ 1	0/ 0
EXCRETA				
-DECREASED DEFECATION	83/18	146/21	179/19	147/22
-FECES SMALL	7/ 5	3/ 2	1/ 1	3/ 2

1- 0 MG/KG/DAY 2- 10 MG/KG/DAY 3- 20 MG/KG/DAY 4- 35 MG/KG/DAY

----- F E M A L E -----

TABLE RANGE:	10-23-09 TO 11-20-09			
GROUP:	1	2	3	4
EXCRETA				
-SOFT STOOL	12/ 9	8/ 6	3/ 3	1/ 1
-WET RED MATERIAL FOUND IN CAGE PAN	0/ 0	1/ 1	0/ 0	0/ 0
-WET RED MATERIAL FOUND ON CAGE FLOOR	0/ 0	1/ 1	0/ 0	0/ 0

1- 0 MG/KG/DAY 2- 10 MG/KG/DAY 3- 20 MG/KG/DAY 4- 35 MG/KG/DAY

a Data obtained from pages 35-37 in the study report.

2. <u>Body weight</u>: There were no statistically significant test substance-related effects on mean body weights at any dose level throughout the gestation treatment period (gestation days 7-29). The decreases in body weight were 3.5% or less and not adverse. Statistically significantly lower mean body weight gains were noted during gestation days 13-21 in the 20 and 35 mg/kg/day groups, relative to the control group, but mean body weight gain in the 35 mg/kg/day group was significantly higher than the control group during gestation days 21-29. As a result, mean body weight gains when the overall gestation treatment period was evaluated (gestation days 7-29) and mean body weights throughout the gestation treatment period were similar in all treated groups when compared to the control group. Due to the transient nature of these weight gain differences, they were not considered evidence of an adverse effect. Mean net body weights, net body weight losses, and gravid uterine weights were similar to the control group, with no statistically significant differences noted.

Table IIA 5.6.11/02–2: Maternal body weight and body weight gain (g; mean±SD)[a] (% change from controls)

GROUP:	0 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY	35 MG/KG/DAY
DAY 0				
MEAN	3554.	3546.	3548.	3524.
% DIFFERENCE		-0.2	-0.2	-0.8
S.D.	244.7	296.7	313.1	236.1
S.E.	49.9	61.9	63.9	47.2
N	24	23	24	25
DAY 4				
MEAN	3448.	3461.	3429.	3436.
% DIFFERENCE		0.4	-0.6	-0.3
S.D.	205.5	285.4	280.4	225.2
S.E.	41.9	59.5	57.2	45.0
N	24	23	24	25
DAY 7				
MEAN	3634.	3632.	3594.	3605.
% DIFFERENCE		-0.1	-1.1	-0.8
S.D.	214.5	296.8	292.3	221.7
S.E.	43.8	61.9	59.7	44.3
N	24	23	24	25
DAY 8				
MEAN	3659.	3661.	3614.	3625.
% DIFFERENCE		0.1	-1.2	-0.9
S.D.	214.7	331.3	310.1	228.9
S.E.	43.8	69.1	63.3	45.8
N	24	23	24	25
DAY 9				
MEAN	3680.	3673.	3634.	3628.
% DIFFERENCE		-0.2	-1.3	-1.4
S.D.	221.6	340.5	318.7	229.3
S.E.	45.2	71.0	65.1	45.9
N	24	23	24	25

GROUP:	0 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY	35 MG/KG/DAY
DAY 10				
MEAN	3693.	3694.	3647.	3641.
% DIFFERENCE		0.0	-1.2	-1.4
S.D.	227.4	360.5	320.6	225.2
S.E.	46.4	75.2	65.4	45.0
N	24	23	24	25
DAY 11				
MEAN	3701.	3708.	3674.	3654.
% DIFFERENCE		0.2	-0.7	-1.3
S.D.	230.1	371.0	326.0	226.3
S.E.	47.0	77.4	66.6	45.3
N	24	23	24	25
DAY 12				
MEAN	3719.	3718.	3685.	3664.
% DIFFERENCE		0.0	-0.9	-1.5
S.D.	226.5	369.2	324.5	229.1
S.E.	46.2	77.0	66.2	45.8
N	24	23	24	25
DAY 13				
MEAN	3737.	3735.	3708.	3690.
% DIFFERENCE		-0.1	-0.8	-1.3
S.D.	226.3	379.7	337.4	235.6
S.E.	46.2	79.2	68.9	47.1
N	24	23	24	25
DAY 14				
MEAN	3770.	3751.	3736.	3718.
% DIFFERENCE		-0.5	-0.9	-1.4
S.D.	231.0	388.2	339.2	223.5
S.E.	47.2	80.9	69.2	44.7
N	24	23	24	25

GROUP:	0 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY	35 MG/KG/DAY
DAY 15				
MEAN	3798.	3770.	3748.	3741.
% DIFFERENCE		-0.7	-1.3	-1.5
S.D.	246.6	394.8	340.3	225.7
S.E.	50.3	82.3	69.5	45.1
N	24	23	24	25
DAY 16				
MEAN	3822.	3799.	3763.	3756.
% DIFFERENCE		-0.6	-1.5	-1.7
S.D.	262.4	405.5	354.6	232.0
S.E.	53.6	84.5	72.4	46.4
N	24	23	24	25
DAY 17				
MEAN	3851.	3818.	3784.	3771.
% DIFFERENCE		-0.9	-1.7	-2.1
S.D.	264.4	415.8	357.6	255.1
S.E.	54.0	86.7	73.0	51.0
N	24	23	24	25
DAY 18				
MEAN	3889.	3839.	3811.	3782.
% DIFFERENCE		-1.3	-2.0	-2.8
S.D.	268.6	423.2	368.7	262.2
S.E.	54.8	88.2	75.3	52.4
N	24	23	24	25
DAY 19				
MEAN	3911.	3863.	3824.	3801.
% DIFFERENCE		-1.2	-2.2	-2.8
S.D.	276.6	423.7	351.0	269.7
S.E.	56.5	88.3	71.7	53.9
N	24	23	24	25

GROUP:	0 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY	35 MG/KG/DAY
DAY 20				
MEAN	3934.	3890.	3837.	3809.
% DIFFERENCE		-1.1	-2.5	-3.2
S.D.	278.6	429.5	377.0	264.6
S.E.	56.9	89.6	77.0	52.9
N	24	23	24	25
DAY 21				
MEAN	3964.	3903.	3857.	3837.
% DIFFERENCE		-1.5	-2.7	-3.2
S.D.	282.5	425.7	389.9	264.5
S.E.	57.7	88.8	79.6	52.9
N	24	23	24	25
DAY 22				
MEAN	3982.	3925.	3879.	3853.
% DIFFERENCE		-1.4	-2.6	-3.2
S.D.	274.3	430.7	395.5	246.7
S.E.	56.0	89.8	80.7	49.3
N	24	23	24	25
DAY 23				
MEAN	4002.	3938.	3881.	3861.
% DIFFERENCE		-1.6	-3.0	-3.5
S.D.	259.5	440.0	385.4	242.1
S.E.	53.0	91.7	78.7	48.4
N	24	23	24	25
DAY 24				
MEAN	4010.	3934.	3886.	3876.
% DIFFERENCE		-1.9	-3.1	-3.3
S.D.	251.2	437.6	374.4	252.0
S.E.	51.3	91.2	76.4	50.4
N	24	23	24	25

GROUP:	0 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY	35 MG/KG/DAY
DAY 25				
MEAN	3987.	3930.	3883.	3881.
% DIFFERENCE		-1.4	-2.6	-2.7
S.D.	239.6	427.1	367.5	252.5
S.E.	48.9	89.1	75.0	50.5
N	24	23	24	25
DAY 26				
MEAN	3945.	3935.	3871.	3883.
% DIFFERENCE		-0.3	-1.9	-1.6
S.D.	232.1	406.5	359.1	256.0
S.E.	47.4	84.8	73.3	51.2
N	24	23	24	25
DAY 27				
MEAN	3921.	3930.	3855.	3884.
% DIFFERENCE		0.2	-1.7	-0.9
S.D.	227.1	389.7	354.5	264.4
S.E.	46.4	81.3	72.4	52.9
N	24	23	24	25
DAY 28				
MEAN	3903.	3928.	3856.	3893.
% DIFFERENCE		0.6	-1.2	-0.3
S.D.	222.4	363.8	348.2	274.9
S.E.	45.4	75.9	71.1	55.0
N	24	23	24	25
DAY 29				
MEAN	3908.	3925.	3850.	3905.
% DIFFERENCE		0.4	-1.5	-0.1
S.D.	236.0	348.6	330.4	280.4
S.E.	48.2	72.7	67.4	56.1
N	24	23	24	25

STATISTICS PERFORMED USING AN ANOVA AND DUNNETT'S TEST
None significantly different from control group
NONGRAVID WEIGHT(S) NOT INCLUDED IN CALCULATION OF MEAN

a Data obtained from pages 39-43 in the study report.

3. <u>Food consumption</u>: Mean maternal food consumption (g/animal/day) was not adverse by test substance administration throughout the gestation treatment period (gestation days 7-29). Statistically significant differences from the control group were intermittent and/or did not occur in a dose-related manner. In addition, there were no consistent trends as both increases and decreases from the control group were noted. The overall decrease in food consumption from day 7-29 was at maximum of 7.6% and not considered to be adverse.

Table IIA 5.6.11/02–3: Food consumption (g/animal/day)[a]

	GROUP:	0 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY	35 MG/KG/DAY
DAY 4- 7	MEAN	168.	178.	160.	168.
	S.D.	23.2	30.0	29.5	21.5
	S.E.	4.7	6.3	6.0	4.3
	N	24	23	24	25
DAY 7- 8	MEAN	181.	189.	178.	178.
	S.D.	35.3	43.8	38.1	29.7
	S.E.	7.2	9.1	7.8	5.9
	N	24	23	24	25
DAY 8- 9	MEAN	178.	180.	166.	161.
	S.D.	34.7	34.9	29.2	24.1
	S.E.	7.1	7.3	6.0	4.8
	N	24	23	24	25
DAY 9- 10	MEAN	178.	181.	169.	162.
	S.D.	27.2	37.9	29.4	34.5
	S.E.	5.5	7.9	6.0	6.9
	N	24	23	24	25
DAY 10- 11	MEAN	171.	173.	167.	153.
	S.D.	30.7	39.6	31.6	26.9
	S.E.	6.3	8.3	6.4	5.4
	N	24	23	24	25

	GROUP:	0 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY	35 MG/KG/DAY
DAY 11- 12	MEAN	167.	171.	157.	148.
	S.D.	31.9	39.1	30.5	37.8
	S.E.	6.5	8.3	6.2	7.6
	N	24	22	24	25
DAY 12- 13	MEAN	157.	155.	147.	136.
	S.D.	39.8	42.4	42.0	47.0
	S.E.	8.1	9.0	8.6	9.4
	N	24	22	24	25
DAY 13- 14	MEAN	156.	140.	145.	140.
	S.D.	45.8	59.5	53.6	49.3
	S.E.	9.3	12.4	10.9	9.9
	N	24	23	24	25
DAY 14- 15	MEAN	157.	140.	134.	139.
	S.D.	51.5	62.4	62.8	55.0
	S.E.	10.5	13.0	12.8	11.0
	N	24	23	24	25
DAY 15- 16	MEAN	162.	156.	144.	144.
	S.D.	69.8	61.0	65.9	55.0
	S.E.	14.6	12.7	13.5	11.0
	N	23	23	24	25
DAY 16- 17	MEAN	177.	162.	144.	154.
	S.D.	73.7	58.2	68.1	54.5
	S.E.	15.0	12.1	14.5	10.9
	N	24	23	22	25
DAY 17- 18	MEAN	180.	171.	166.	160.
	S.D.	60.3	55.1	51.6	58.4
	S.E.	12.3	11.5	10.5	11.7
	N	24	23	24	25
DAY 18- 19	MEAN	181.	168.	162.	154.
	S.D.	61.6	49.1	49.9	50.3
	S.E.	12.6	10.2	10.4	10.3
	N	24	23	23	24
DAY 19- 20	MEAN	182.	175.	157.	155.
	S.D.	55.6	42.7	62.9	48.4
	S.E.	11.6	8.9	13.1	9.7
	N	23	23	23	25
DAY 20- 21	MEAN	180.	163.	153.	150.
	S.D.	47.3	44.5	53.3	48.7
	S.E.	9.7	9.7	11.1	9.9
	N	24	21	23	24

GROUP:	0 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY	35 MG/KG/DAY
DAY 21- 22				
MEAN	178.	155.	138.**	143.*
S.D.	33.3	43.1	54.8	38.5
S.E.	7.0	9.2	11.4	7.7
N	23	22	23	25
DAY 22- 23				
MEAN	157.	134.	122.*	129.
S.D.	39.3	58.3	50.8	39.8
S.E.	8.0	12.4	10.6	8.0
N	24	22	23	25
DAY 23- 24				
MEAN	130.	107.	104.	116.
S.D.	41.9	57.4	57.3	40.7
S.E.	8.6	12.2	12.0	8.1
N	24	22	23	25
DAY 24- 25				
MEAN	81.	86.	86.	98.
S.D.	52.5	48.1	47.5	44.3
S.E.	10.7	10.0	10.1	8.9
N	24	23	22	25
DAY 25- 26				
MEAN	58.	75.	71.	88.
S.D.	49.7	38.3	48.3	44.9
S.E.	10.2	8.2	10.1	9.0
N	24	22	23	25

STATISTICS PERFORMED USING AN ANOVA AND DUNNETT'S TEST
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test
NONGRAVID WEIGHT(S) NOT INCLUDED IN CALCULATION OF MEAN

GROUP:	0 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY	35 MG/KG/DAY
DAY 26- 27				
MEAN	48.	70.	65.	91.**
S.D.	40.7	41.9	51.1	43.4
S.E.	8.3	8.7	10.6	8.7
N	24	23	23	25
DAY 27- 28				
MEAN	53.	82.	71.	101.**
S.D.	41.3	48.6	49.3	45.9
S.E.	8.4	10.1	10.1	9.2
N	24	23	24	25
DAY 28- 29				
MEAN	65.	84.	75.	96.
S.D.	43.8	52.1	49.0	53.5
S.E.	9.0	10.9	10.0	10.7
N	24	23	24	25
DAY 7- 10				
MEAN	179.	184.	171.	167.
S.D.	29.6	37.5	30.8	25.6
S.E.	6.0	7.8	6.3	5.1
N	24	23	24	25
DAY 10- 13				
MEAN	165.	167.	157.	145.
S.D.	30.9	38.9	32.5	35.3
S.E.	6.3	8.3	6.6	7.1
N	24	22	24	25

STATISTICS PERFORMED USING AN ANOVA AND DUNNETT'S TEST
** = Significantly different from the control group at 0.01 using Dunnett's test
NONGRAVID WEIGHT(S) NOT INCLUDED IN CALCULATION OF MEAN

GROUP:	0 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY	35 MG/KG/DAY
DAY 13- 21				
MEAN	172.	160.	151.	150.
S.D.	52.0	50.9	49.6	47.1
S.E.	10.6	10.6	10.3	9.4
N	24	23	23	25
DAY 21- 29				
MEAN	96.	99.	93.	108.
S.D.	30.5	32.8	39.9	32.3
S.E.	6.2	7.0	8.3	6.5
N	24	22	23	25
DAY 7- 29				
MEAN	144.	142.	133.	136.
S.D.	24.8	33.7	32.7	24.5
S.E.	5.1	7.0	6.7	4.9
N	24	23	24	25

STATISTICS PERFORMED USING AN ANOVA AND DUNNETT'S TEST
None significantly different from control group
NONGRAVID WEIGHT(S) NOT INCLUDED IN CALCULATION OF MEAN

a Data extracted from pages 52-57 of the study report

4. Sacrifice and Pathology:

a) Gross Pathology: At the scheduled necropsy on gestation day 29, no test substance-related adverse internal findings were observed. Macroscopic findings observed in the test substance-treated groups occurred infrequently, at similar frequencies in the control group, and/or in a manner that was not dose-related. The only statistically significant difference from the control group was a decrease in the incidence of females with accessory spleen in the 20 mg/kg/day group; however, it was not dose responsive and not considered adverse. Two females were non-gravid in the low-dose group, however the effect was not dose responsive.

Table IIA 5.6.11/02–4a: Gross Pathology[a]

GROUP :	1	2	3	4
NUMBER EXAMINED	1	2	1	0
NONGRAVID -- AMMONIUM SULFIDE NEGATIVE	1	2	1	0
OVIDUCTS: CYST(S)	0	1	0	0
SPLEEN: ACCESSORY	0	2	0	0

1- 0 MG/KG/DAY 2- 10 MG/KG/DAY 3- 20 MG/KG/DAY 4- 35 MG/KG/DAY

GROUP :	1	2	3	4
NUMBER EXAMINED	24	23	24	25
NO SIGNIFICANT CHANGES OBSERVED	13	11	18	14
OVIDUCTS: CYST(S)	6	8	5	9
LIVER: PALE	1	1	1	1
SPLEEN: ACCESSORY	5	2	0*	5
KIDNEYS: CONTENTS, THICK YELLOW	1	0	0	0
KIDNEYS: SMALL	1	0	0	0
LUNGS: AREA(S), DARK RED	1	3	0	0
SKIN: HAIR LOSS	0	0	1	0
HEART: AREA(S), WHITE	0	1	0	0
UTERUS: AREA(S), DARK RED	0	1	0	0
LUNGS: DISCOLORATION, DARK RED	1	1	0	0
LIVER: MOTTLED	0	0	1	0

1- 0 MG/KG/DAY 2- 10 MG/KG/DAY 3- 20 MG/KG/DAY 4- 35 MG/KG/DAY
* = Significantly different from the control group at 0.05 using 2-tailed Fisher's Exact test

a Data extracted from pages 58-59 of the study report

b) Liver weights: Mean absolute liver weights and liver weights adjusted for net body weight in the 10, 20, and 35 mg/kg/day groups were similar to the control group. The maximum absolute liver weight changed by a maximum of 5.4%, thus not adverse.

Table IIA 5.6.11/02–4b: Mean liver weight (mean±SD)[b]

Sex	Organ	Statistic	Dosage Level (mg/kg/day) 0	10	20	35
F	Liver Weight	Mean	91.41	96.33	95.19	94.95
		% Difference Control		5.38	4.14	3.87
		LSMean	90.27	95.42	96.85	95.31
		SD	13.740	19.816	17.894	13.696
		SE	2.805	4.132	3.653	2.796
		N	24	23	24	24
		Dunnett p-value#		0.301	0.133	0.310
	Net Body Weight - a	Mean	3412.7	3406.9	3342.6	3375.2
		SD	218.56	369.96	313.63	254.93
		SE	44.61	77.14	64.02	52.04
		N	24	23	24	24
	Liver to Net Body Weight Ratio - a	Mean	2.7	2.8	2.8	2.8
		SD	0.38	0.34	0.44	0.28
		SE	0.08	0.07	0.09	0.06
		N	24	23	24	24

STATISTICS PERFORMED USING AN ANCOVA AND DUNNETT'S TEST, WITH NET BODY WEIGHT AS A COVARIATE
: Level of significance tested = .05. * : Statistically significant.
LSMean is adjusted liver weight
a = Net Body Weight and Liver to Net Body Weight Ratio were not statistically analyzed.

b Data extracted from pages 60-61 of the study report

5. <u>Caesarean section data</u>: Intrauterine growth and survival were not affected by test substance administration with statistical significance. Although the mean litter proportion of late resorptions in the 35 mg/kg/day group (2.5% per litter) was significantly higher than the concurrent control group (0.0% per litter), this value was within the range of values in the WIL historical control data (up to 5.1% per litter). There was no significant increase in the overall post-implantation loss in the 35 mg/kg/day group. Other parameters evaluated (post-implantation loss, live litter size, mean fetal body weights, fetal sex ratios, mean numbers of corpora lutea, and implantation sites and the mean litter proportions of pre implantation loss) were similar across all groups.

Table IIA 5.6.11/02–5: Caesarean Section Observations[a]

GROUP	SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1 TOTAL	107	114	221	0	9	0	9	230	250	20	NA	24
MEAN	4.5	4.8	9.2	0.0	0.4	0.0	0.4	9.6	10.4	0.8	39.7	
S.D.	1.28	1.57	1.32	0.00	0.58	0.00	0.58	1.25	1.69	0.92	5.26	
S.E.	0.26	0.32	0.27	0.00	0.12	0.00	0.12	0.25	0.35	0.19	1.07	
2 TOTAL	104	108	212	0	5	3	8	220	244	24	NA	23
MEAN	4.5	4.7	9.2	0.0	0.2	0.1	0.3	9.6	10.6	1.0	40.5	
S.D.	1.44	1.61	1.41	0.00	0.52	0.34	0.57	1.53	1.73	1.36	4.17	
S.E.	0.30	0.34	0.29	0.00	0.11	0.07	0.12	0.32	0.36	0.28	0.87	
3 TOTAL	116	109	225	0	5	4	9	234	259	25	NA	24
MEAN	4.8	4.5	9.4	0.0	0.2	0.2	0.4	9.8	10.8	1.0	39.3	
S.D.	2.26	1.74	2.18	0.00	0.51	0.38	0.71	2.23	2.40	1.68	4.78	
S.E.	0.46	0.36	0.45	0.00	0.10	0.08	0.15	0.46	0.49	0.34	0.98	
4 TOTAL	115	114	229	1	6	7	14	243	258	15	NA	25
MEAN	4.6	4.6	9.2	0.0	0.2	0.3	0.6	9.7	10.3	0.6	41.9	
S.D.	1.50	1.71	1.75	0.20	0.52	0.61	0.77	1.86	1.65	1.15	5.89	
S.E.	0.30	0.34	0.35	0.04	0.10	0.12	0.15	0.37	0.33	0.23	1.18	

STATISTICS PERFORMED USING AN ANOVA AND DUNNETT'S TEST
None significantly different from control group
NA = NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

1- 0 MG/KG/DAY 2- 10 MG/KG/DAY 3- 20 MG/KG/DAY 4- 35 MG/KG/DAY

Sex	Parameter	Value	Statistic	Dosage Level (mg/kg/day) 0	10	20	35
F	Dead Fetuses	Litter Based Mean Percentage	Mean	0.0	0.0	0.0	0.4
		Double Arcsine Transformation	Mean	0.3	0.3	0.3	0.3
			SD	0.02	0.02	0.04	0.09
			SE	0.00	0.00	0.01	0.02
			N	24	23	24	25
			Dunnett p-value#		1.000	0.998	0.481
	Early Resorptions	Litter Based Mean Percentage	Mean	3.9	2.2	2.1	2.6
		Double Arcsine Transformation	Mean	0.5	0.4	0.4	0.4
			SD	0.24	0.20	0.19	0.22
			SE	0.05	0.04	0.04	0.04
			N	24	23	24	25
			Dunnett p-value#		0.526	0.477	0.654
	Late Resorptions	Litter Based Mean Percentage	Mean	0.0	1.2	1.5	2.5
		Double Arcsine Transformation	Mean	0.3	0.4	0.4	0.4
			SD	0.02	0.14	0.16	0.20
			SE	0.00	0.03	0.03	0.04
			N	24	23	24	25
			Dunnett p-value#		0.420	0.219	0.044*
	Percent Females	Litter Based Mean Percentage	Mean	51.2	50.5	49.5	49.1
		Double Arcsine Transformation	Mean	1.6	1.6	1.6	1.6
			SD	0.29	0.27	0.32	0.29
			SE	0.06	0.06	0.07	0.06
			N	24	23	24	25
			Dunnett p-value#		0.997	0.954	0.945

: Level of significance tested = .05. * : Statistically significant.

Sex	Parameter	Value	Statistic	Dosage Level (mg/kg/day) 0	10	20	35
F	Percent Males	Litter Based Mean Percentage	Mean	48.8	49.5	50.5	50.9
		Double Arcsine Transformation	Mean	1.5	1.6	1.6	1.6
			SD	0.29	0.27	0.32	0.29
			SE	0.06	0.06	0.07	0.06
			N	24	23	24	25
			Dunnett p-value#		0.997	0.954	0.945
	Post-Implantation Loss	Litter Based Mean Percentage	Mean	3.9	3.4	3.6	5.4
		Double Arcsine Transformation	Mean	0.5	0.5	0.5	0.5
			SD	0.24	0.22	0.25	0.27
			SE	0.05	0.05	0.05	0.05
			N	24	23	24	25
			Dunnett p-value#		0.992	0.982	0.794
	Pre-Implantation Loss	Litter Based Mean Percentage	Mean	7.3	9.1	8.7	5.7
		Double Arcsine Transformation	Mean	0.6	0.6	0.6	0.5
			SD	0.27	0.34	0.39	0.33
			SE	0.06	0.07	0.08	0.07
			N	24	23	24	25
			Dunnett p-value#		0.976	1.000	0.705
	Total Resorptions	Litter Based Mean Percentage	Mean	3.9	3.4	3.6	5.1
		Double Arcsine Transformation	Mean	0.5	0.5	0.5	0.5
			SD	0.24	0.22	0.25	0.26
			SE	0.05	0.05	0.05	0.05
			N	24	23	24	25
			Dunnett p-value#		0.991	0.981	0.866

: Level of significance tested = .05. * : Statistically significant.

Sex	Parameter	Value	Statistic	Dosage Level (mg/kg/day) 0	10	20	35
F	Viable Fetuses	Litter Based Mean Percentage	Mean	96.1	96.6	96.4	94.6
		Double Arcsine Transformation	Mean	2.7	2.7	2.7	2.6
			SD	0.24	0.22	0.25	0.27
			SE	0.05	0.05	0.05	0.05
			N	24	23	24	25
			Dunnett p-value#		0.992	0.982	0.794

: Level of significance tested = .05. * : Statistically significant.

GROUP:	0 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY	35 MG/KG/DAY
MALE FETAL WEIGHTS (g)				
MEAN	40.1	41.8	39.6	42.0
% DIFFERENCE		4.2	-1.2	4.7
S.D.	5.81	4.41	5.26	6.56
S.E.	1.19	0.92	1.07	1.31
N	24	23	24	25
FEMALE FETAL WEIGHTS (g)				
MEAN	39.3	39.3	39.0	42.0
% DIFFERENCE		0.0	-0.8	6.9
S.D.	5.33	5.12	4.85	5.69
S.E.	1.09	1.07	0.99	1.14
N	24	23	24	25
COMBINED FETAL WEIGHTS (g)				
MEAN	39.7	40.5	39.3	41.9
% DIFFERENCE		2.0	-1.0	5.5
S.D.	5.26	4.17	4.78	5.89
S.E.	1.07	0.87	0.98	1.18
N	24	23	24	25

STATISTICS PERFORMED USING AN ANOVA AND DUNNETT'S TEST
None significantly different from control group

a Data obtained from pages 62-66 in the study report.

B. Developmental toxicity:

1. External examinations: One fetus in the 10 mg/kg/day group had microphthalmia with no apparent skeletal origin. The effect was not dose responsive and was not attributed to test substance administration. There were no other external malformations nor developmental variations observed in fetuses in this study.

Table IIA 5.6.11/02–6: External Examinations[a]

DOSE GROUP:		1	2	3	4
NUMBER OF LITTERS EXAMINED EXTERNALLY		24	23	24	25
MICROPHTHALMIA AND/OR ANOPHTHALMIA	MEAN	0.0	0.4	0.0	0.0
	S.D.	0.00	2.09	0.00	0.00
	S.E.	0.00	0.43	0.00	0.00

1- 0 MG/KG/DAY 2- 10 MG/KG/DAY 3- 20 MG/KG/DAY 4- 35 MG/KG/DAY

a Data extracted from page 68 of the study report.

2. Visceral examinations: A total of 2 fetuses were observed with visceral malformations. One fetus in the 10 mg/kg/day group had a diaphragmatic hernia consisting of a portion of the median and left lobes of the liver protruding into the thoracic cavity through an opening in the diaphragm. This finding was not attributed to test substance administration since it was not dose responsive. One fetus in the control group had lobular agenesis of the lungs (right accessory lobe absent). Visceral developmental variations noted in the test substance-treated groups occurred in a frequency similar to the control group and/or in a manner that was not dose-related. The numbers of fetuses with accessory spleen(s) in the 10, 20, and 35 mg/kg/day groups were statistically significantly lower than the concurrent control group; however, not considered adverse. Other findings were not statistically significantly different from the

control group and the mean litter proportions were within the ranges of the WIL historical control data. A distended stomach was noted for fetuses in the 10 and 20 mg/kg/day groups, respectively. A depressed area in the cranium (no apparent skeletal origin) was noted for one fetus in the 35 mg/kg/day group. These findings were not considered to be test article-related.

Table IIA 5.6.11/02–7: Visceral Examinations[a]

DOSE GROUP:		1	2	3	4
NUMBER OF LITTERS EXAMINED VISCERALLY		24	23	24	25
DIAPHRAGMATIC HERNIA	MEAN	0.0	0.5	0.0	0.0
	S.D.	0.00	2.32	0.00	0.00
	S.E.	0.00	0.48	0.00	0.00
LUNGS- LOBULAR AGENESIS	MEAN	0.4	0.0	0.0	0.0
	S.D.	2.04	0.00	0.00	0.00
	S.E.	0.42	0.00	0.00	0.00

1- 0 MG/KG/DAY 2- 10 MG/KG/DAY 3- 20 MG/KG/DAY 4- 35 MG/KG/DAY

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	221	212	225	229	24	23	24	25
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	221	212	225	229	24	23	24	25
MAJOR BLOOD VESSEL VARIATION	28	15	17	20	10	8	10	8
LIVER- ACCESSORY LOBULE(S)	1	0	0	1	1	0	0	1
ACCESSORY SPLEEN(S)	55	31**	29**	37*	22	13**	16	16*
HEART- EXTRA PAPILLARY MUSCLE	14	13	12	15	8	11	9	10
LUNGS- SMALL	1	0	0	0	1	0	0	0
GALLBLADDER- ABSENT OR SMALL	5	12	2	4	5	7	2	4
RETROCAVAL URETER	3	6	0	2	3	4	0	2
HEMORRHAGIC RING AROUND THE IRIS	1	1	2	1	1	1	1	1
HEART- SMALL	0	1	0	0	0	1	0	0
GALLBLADDER- BILOBED	1	1	0	0	1	1	0	0
SPLEEN- SMALL	0	1	1	0	0	1	1	0
NUMBER EXAMINED SKELETALLY	221	212	225	229	24	23	24	25
13TH RUDIMENTARY RIB(S)	39	36	37	41	18	20	18	21
13TH FULL RIB(S)	72	67	56	73	18	19	20	23
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	18	18	29	27	9	7	12	10
HYOID ARCH(ES) BENT	14	8	21	12	7	7	9	6
27 PRESACRAL VERTEBRAE	13	11	13	16	6	6	6	9
STERNEBRAE WITH THREAD-LIKE ATTACHMENT	5	4	1	2	4	2	1	2
25 PRESACRAL VERTEBRAE	0	1	0	2	0	1	0	2
7TH CERVICAL RIB(S)	3	2	1	1	2	2	1	1
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	1	1	2	1	1	1	2	1
EXTRA SITE OF OSSIFICATION ANTERIOR TO STERNEBRA #1	3	5	1	1	1	2	1	1
ACCESSORY SKULL BONE(S)	0	1	0	1	0	1	0	1

1- 0 MG/KG/DAY 2- 10 MG/KG/DAY 3- 20 MG/KG/DAY 4- 35 MG/KG/DAY

* = Significantly different from the control group at 0.05 using Fisher's Exact test (2-TAILED)
** = Significantly different from the control group at 0.01 using Fisher's Exact test (2-TAILED)

a Data extracted from pages 69, 72 of the study report.

3. <u>Skeletal examinations</u>: No test substance-related skeletal malformations were noted for fetuses in this study with statistical significance. Skeletal malformations were observed in 3(2), 1(1), 2(2), and 2(2) fetuses (litters) from the control, 10, 20, and 35 mg/kg/day groups, respectively. Fused sternebrae were seen in single fetuses from the control, 10 mg/kg/day, and 35 mg/kg/day dose groups. The control group fetus also exhibited a vertebral centra anomaly, as did a litter mate. One additional control fetus exhibited a costal cartilage anomaly. In the 20 mg/kg/day dose group, malformations were observed in two fetuses, one with vertebral anomaly with associated rib anomaly and the other with malaligned sternebrae. At 35 mg/kg/day, 1 fetus exhibited a rib anomaly. Findings in the test substance-treated groups occurred infrequently (single fetuses), in similar frequencies in the concurrent control group, and/or in a manner that was not dose-related, and were therefore considered unrelated to treatment and not adverse. Skeletal variations noted in the test substance-treated groups occurred in a frequency similar to the control group and/or in a manner that was not dose-related.

Findings were not statistically significantly different from the control group and the mean litter proportions were within the ranges of values in the WIL historical control data.

Table IIA 5.6.11/02–8: Skeletal Examinations[a]

DOSE GROUP:		1	2	3	4
NUMBER OF LITTERS EXAMINED SKELETALLY		24	23	24	25
RIB ANOMALY	MEAN	0.0	0.0	0.0	0.3
	S.D.	0.00	0.00	0.00	1.67
	S.E.	0.00	0.00	0.00	0.33
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	MEAN	0.0	0.0	0.4	0.0
	S.D.	0.00	0.00	2.04	0.00
	S.E.	0.00	0.00	0.42	0.00
STERNEBRAE FUSED	MEAN	0.3	0.5	0.0	0.4
	S.D.	1.70	2.32	0.00	2.00
	S.E.	0.35	0.48	0.00	0.40
STERNEBRA(E) MALALIGNED (SEVERE)	MEAN	0.0	0.0	0.4	0.0
	S.D.	0.00	0.00	1.86	0.00
	S.E.	0.00	0.00	0.38	0.00
COSTAL CARTILAGE ANOMALY	MEAN	0.4	0.0	0.0	0.0
	S.D.	2.04	0.00	0.00	0.00
	S.E.	0.42	0.00	0.00	0.00
VERTEBRAL CENTRA ANOMALY	MEAN	0.7	0.0	0.0	0.0
	S.D.	3.40	0.00	0.00	0.00
	S.E.	0.69	0.00	0.00	0.00

1- 0 MG/KG/DAY 2- 10 MG/KG/DAY 3- 20 MG/KG/DAY 4- 35 MG/KG/DAY

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED SKELETALLY	221	212	225	229	24	23	24	25
REDUCED OSSIFICATION OF THE SKULL	1	0	0	0	1	0	0	0
REDUCED OSSIFICATION OF THE 12TH RIB(S)	0	0	0	1	0	0	0	1

1- 0 MG/KG/DAY 2- 10 MG/KG/DAY 3- 20 MG/KG/DAY 4- 35 MG/KG/DAY

None significantly different from control group

a Data extracted from pages 70, 73 of the study report.

III. Discussion

A. <u>Investigator's conclusion (extracted from page 30 in the study report)</u>:
In the current study, the top dose level of 35 mg/kg/day produced only marginal effects on maternal body weight gains that were not considered adverse. However, in the dose range-finding study in pregnant rabbits (Sawhney Coder, 2011, WIL-639039), decreased maternal body weight gain was observed throughout the majority of gestation at a dose of 50 mg/kg/day, and 2 of 10 animals (20%) were euthanized due to excessive weight loss, and a third animal aborted late in gestation. In light of the effects observed in this range-finding study, a top dose of 35 mg/kg/day was considered to be appropriate for the guideline-driven study. Based on the lack of maternal or embryo/fetal developmental toxicity, a dose level of 35 mg/kg/day (the highest dose level evaluated) was considered to be the no-observed adverse- effect level (NOAEL) for maternal toxicity and the no-observed-effect level (NOEL) for embryo/fetal developmental toxicity when SYN545192 was administered orally by gavage to pregnant New Zealand White rabbits.

B. <u>Reviewer's conclusions</u>: The Agency concurs with the NOAEL and LOAEL selections, the highest dose tested was the study NOAEL. The body weight changes were 3.5% or less, suggesting inadequate dosing, however, this rabbit developmental study utilized doses greater than the rat developmental study (i.e. up to 35 and 30 mg/kg/day, respectively). Adverse responses were observed in the rabbit range-finding study at doses

of 50 mg/kg/day (i.e. decreased body weight gain, excessive body weight decreases and abortion, thus this dose can be considered to be a rabbit developmental study LOAEL.

C. Deficiencies: Some of the dosing solutions were out of specifications, however, it was not expected to affect the outcome of the study.

D. References:

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Acute Neurotoxicity Study– Rat

Report:	Sommer E (2011), **SYN545192 - Acute Oral (Gavage) Neurotoxicity Study in the Rat,** Harlan Laboratories Ltd., Zelgliweg 1, CH-4452 Itingen / Switzerland. Laboratory Report No. C79992. Issue date 12 May, 2011. Unpublished. MRID #48604455

Guidelines: Acute Neurotoxicity Study. OECD 424 (1997): U.S. EPA OPPTS 870.6200 (1998): EU Council Directive 67/548/EEC B.43 (2000): JMAFF Notification No. 12 NohSan 8147 (2000)

Sponsor: Syngenta Crop Protection, LLC, 410 Swing Road, Post Office Box 18300 Greensboro, NC 27419-8300 USA

Executive Summary:

In this acute neurotoxicity study, HanRcc:Wistar (SPF) rats (10 rats/sex/group) were administered single oral gavage doses of SYN545192 (97.0% purity, prepared in 0.5% aqueous carboxymethylcellulose vehicle) at dose levels of 0 (control), 10, 30 and 80 mg/kg, respectively, and observed for 15 days. Daily cage side observations were performed on all rats prior to study start, on day 1 (at approximately 1 hour after dose administration), and once daily thereafter. Detailed clinical observations, comprising open field evaluation of clinical signs, were performed in a randomized order once prior to study start, on day 1 (at approximately 5-hours post-dose) and on days 7 and 14. Functional observation battery (FOB) evaluations were performed once prior to study start, on day 1 (approximately 2 to 4 hours after dose administration), and on days 7 and 14. Functional observation battery (FOB) evaluations including quantitative assessment of landing foot splay, body temperature, sensory perception and muscle weakness, locomotor activity (LMA) was assessed over a time period of 30 minutes after each FOB evaluation. Food consumption was recorded during acclimatization and over days 1 - 2, 8 - 9, and 14 - 15. Body weights were measured during acclimatization and on days 1, 8 and 15. At the end of the scheduled post-dose observation period (day 15), 5 rats/sex/group were perfusion-fixed *in situ,* the brain weights were measured, and selected nervous system tissues were collected, processed and examined microscopically.

Following the administration of a single oral (gavage) dose of SYN545192 to Wistar rats at 10, 30 and 80 mg/kg, evidence of toxicity was limited to the 80 mg/kg males and the 30 and 80 mg/kg females. The majority of treatment-related findings occurred post-dose on day 1, and included multiple clinical observations, decreases in mean body temperature, decreases in locomotor activity parameters, and/or decreases in mean grip strength. Other findings noted for 80 mg/kg males and the 30 and 80 mg/kg females included lower mean food consumption over the first day of treatment (day 1 - 2) with corresponding reductions (slight but not statistically significant) in body weight gains over the first week of treatment (days 1 - 8). All of these treatment-related changes were transient. No clinical and/or behavioral signs of toxicity were evident in any of the rats

after day 2, and mean overall (day 1 - 14) food consumption and body weight gain values were not affected by the treatment. At study termination no treatment-related neurohistopathological lesions were present. Thus, the transient treatment-related findings noted in the 80 mg/kg males and the 30 and 80 mg/kg females were attributed to the systemic toxicity rather than to specific neurotoxic effects.

Based on these findings, the LOAEL (lowest-observed adverse effect level) is 30 mg/kg for females and 80 mg/kg for males. The NOAEL (no-observed-adverse-effect level) for generalized systemic toxicity was 10 mg/kg for females and 30 mg/kg for males. The NOAEL for neurotoxicity was 80 mg/kg for both genders.

The study is classified as **acceptable/guideline** as an acute neurotoxicity study in rats (870.6200).

Compliance: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. Materials:

Test Material:	SYN545192
Description:	Beige powder
Lot/Batch number:	SMU9BP005
Purity:	97.0%
CAS #:	NA
Stability of test compound:	Stable At room temperature (20 ± 5 °C), in the original container, away from direct sunlight. Expiry date 28 Feb, 2013
Vehicle:	0.5% w/v carboxymethylcellulose (CMC), high viscosity grade, in distilled water.
Positive control:	Acrylamide

Test Animals:

Species	Rat
Strain	HanRcc: WIST (SPF)
Age at dosing	7 weeks
Weight at dosing	Males 191.3 to 223.0 g, females 128.8 to 167.0 g
Source	Harlan Laboratories, B.V. Kreuzelweg 53 5961 NM Horst / Netherlands
Housing	Individually in Makrolon type-3 cages
Acclimatization period	1 week
Diet	Pelleted standard Harlan Teklad 2914C rodent maintenance diet, batch no. B0796 (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland) *ad libitum*
Water	Community tap water from Itingen *ad libitum.*
Environmental conditions	Temperature:22±3°C Humidity: 30-70% Air changes: 10-15 changes/hour Photoperiod: 12 hours light/12 hours dark

B. Study Design and Methods:

1. In-life dates: Start: 15 April 2010 End: 06 May 2010 (males), 07 May 2010 (females)

2. Animal assignment and treatment: Animals were assigned to the test groups noted in Table IIA 5.7.1/02-1 by a computer-generated random algorithm program. In this acute neurotoxicity study, HanRcc:Wistar (SPF) rats in groups 1 – 4 (10 rats/sex/group) were administered single oral doses of SYN545192 (prepared in 0.5% aqueous carboxymethylcellulose vehicle) at dose levels of 0 (control), 10, 30 and 80 mg/kg, respectively, and observed for 15 days. General daily cage side observations were performed on all rats prior to study start, on day 1 (at approximately 1 hour after dose administration), and once daily thereafter. Detailed clinical observations, comprising open field evaluation of clinical signs, were performed in a randomized order once prior to study start, on day 1 (at approximately 5-hours post-dose) and on days 7 and 14. Functional observation battery (FOB) evaluations were performed once prior to study start, on day 1 (approximately 2 to 4 hours after dose administration), and on days 7 and 14. Functional observation battery (FOB) evaluations including quantitative assessment of landing foot splay, body temperature, sensory perception and muscle weakness, locomotor activity (LMA) was assessed over a time period of 30 minutes after each FOB evaluation. Food consumption was recorded during acclimatization and over days 1 - 2, 8 - 9, and 14 - 15. Body weights were measured during acclimatization and on days 1, 8 and 15. At the end of the scheduled post-dose observation period (day 15), 5 rats/sex/group were perfusion-fixed *in situ,* the brain weights were measured, and selected nervous system tissues were collected, processed and examined microscopically.

Table IIA 5.7.1/02-1. Study design

Experimental Parameter	Dose Group (mg/kg/day)			
	0	10	30	80
Total number of Animals/sex/group	10/sex	10/sex	10/sex	10/sex
Behavioral Testing (FOB, Motor Activity)	10/sex	10/sex	10/sex	10/sex
Neuropathology	5/sex	5/sex	5/sex	5/sex

3. Dose selection rationale: The dose levels were based on recent data from several studies. In one study (data provided by the Sponsor), 100% mortality (3/3 rats) was noted following a single oral (gavage) dose of 175 mg/kg. At the next lower dose (55 mg/kg) there was 25% mortality (1/4 rats). In a preliminary acute neurotoxicity study (Harlan Laboratories study C79981), no mortality (0/6 rats) was noted at the high dose level of 100 mg/kg. The incidence and severity of post-dose clinical signs occurred in a dose-related manner, and included reduced activity, posture/gait creeping, hunched posture, a decrease in righting response, bizarre behavior, paddling movements, and piloerection. Several of these signs were noted at the low dose of 25 mg/kg. These clinical signs were transient and the animals appeared essentially normal by 24 hours post-dose. Due to the severity of the clinical signs noted in the preliminary study at 100 mg/kg, the high dose used in this acute neurotoxicity study was 80 mg/kg. A variety of post-dose clinical signs were anticipated. It was anticipated that some mortality may occur at this dose level, particular based on the severity of signs noted for the 100 mg/kg females. However, the extent of mortality, if any, was not expected to compromise the integrity of the study. Based on the post-dose clinical observations noted in the preliminary study at 25 mg/kg (including reduced activity, posture/gait creeping, hunched posture, a decrease in righting reflex, abnormal gait, and/or piloerection), the low dose of 10 mg/kg used in this acute neurotoxicity study was anticipated to produce a minimal incidence of post-dose clinical signs. In the preliminary acute neurotoxicity study with SYN545192 (Harlan Laboratories study C79981), the incidence and severity of post-dose clinical signs were generally greatest at 2 - 4 hours after dose administration. Therefore the time to peak effect in this acute neurotoxicity study was designated at 2 - 3 hours post-dose.

4. Dose preparation and analysis: Carboxymethylcellulose sodium salt (CMC, high viscosity) at 5.0006g was weighed and placed into a tared 1 L glass bottle to prepare 1.0 liter of vehicle. Purified water (approximately 800 mL) was added at ambient temperature, and the mixture was stirred overnight using a magnetic stirrer. Afterwards, the mixture was adjusted to a final volume of 1 L with purified water, and the vehicle was stirred for an additional 10 minutes. The prepared vehicle was stored refrigerated (5 ± 3 ºC). Dose formulations (set A and set B) were prepared on 21 April 2010 by weighing defined amounts of SYN545192 and 0.5% (w/v) CMC aqueous solution into a 100 mL calibrated (volumetric) glass container on a suitable precision balance. Following homogenization for at least five minutes by use of an ultra-turrax, the preparations were stirred for 20 minutes using a magnetic stirrer. Samples of approximately 2 g were quantitatively collected from top, middle and bottom of each dose formulation while stirring.

Results: **Homogeneity Analysis:** range was 0.3% to 3.2%

Stability Analysis: Dose formulations prepared at 0.1, 1.0 and 20.0 mg/mL were determined to be stable when stored for 24 hours at room temperature (20 ± 5 °C) under ambient light conditions and for 8 days when stored refrigerated (5 ± 3 °C) (Harlan Laboratories study C80036).

Concentration Analysis: range was 98.4% to 101.9% of target

The analytical data indicated that the mixing procedure was adequate and that the variance between the nominal and actual dosage to the animals was acceptable.

5. Statistics: For statistical analyses the S-plus with SAS (version 9.1.3) and proc glm (for diagnostic homogenicity test of the variance according to Bartlett) and proc mixed (because the Dunnett's test could not be directly released) were applied. The following statistical approaches were used in this study

 All analyses were two-tailed for significance levels of 5% and 1%.

 All means were presented with standard deviations.

 If the variances were clearly heterogeneous, appropriate transformations (e.g. log, square root, double arcsine) were used in an attempt to stabilize the variances. In the final report, any transformations that were utilized were indicated in the specific results tables and/or the statistical methods section.

For quantitative data: Body weights, cumulative body weight gain, food consumption, quantitative FOB measurements (grip strength, landing foot splay, and/or body temperature), locomotor activity data at each measurement interval and overall activity, and absolute organ weights were analyzed initially by a one-way analysis of variance (ANOVA) followed by ANOVA and Dunnett's test. And the adjusted mean organ weights from the ANCOVA were analyzed.

 Organ weights were analyzed by analysis of covariance (ANCOVA) on final body weight (Shirley, 1977). This statistical analysis provided an adjusted organ weight value for the brain weight, which are displayed in the results table along with flags for statistical significance. Organ to body weight ratios were also analyzed by analysis of covariance since this provides a better method of allowing for differences in final body weight (Shirley, 1977).

 Summary values of organ to body weight ratios are presented but these were not analyzed statistically.

 For all of the parameters evaluated initially by ANOVA or ANCOVA, Dunnett's test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA. The Dunnett's test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant, and statistical flags are presented in the tables of results in the final report.

 Macropathology data were analyzed using Fisher's Exact test.

 For qualitative data (e.g. possible values of 0, 1, 2 or present/absent): Qualitative functional observational battery parameters or any other parameters not specifically

mentioned above that yield qualitative data are presented as summary data, but were not be analyzed statistically.

C. Observations

1. Mortality and clinical observations: Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. Observations for viability / mortality were recorded twice daily.

General cage side observations: All animals were observed for clinical signs once during acclimatization, at approximately 1 hour after dose administration on day 1, and once daily thereafter.

Detailed clinical observations: The animals were observed in their home cages, outside their home cages in a standard arena and in the hand. These observations were performed in random sequence once before commencement of administration, at approximately 5 hours after dosing on day 1, and on days 8 and 15.

2. Body weights: Body weights were recorded once during the acclimatization period and on days 1, 8 and 15 thereafter.

3. Food consumption: Food consumption was recorded over a 24-hour period at the following intervals: once during the acclimatization period, and on days 1 - 2, 8 - 9, and 14 - 15.

4. Neurobehavioral assessment

a. Functional observational battery (FOB): A FOB was performed for all animals once during acclimatization, on day 1 (at time of peak effect, approximately 2 to 4 hours after dosing), on day 7, and on day 14. The FOB included a quantitative assessment of landing foot splay, sensory perception and muscle weakness. For the conduct of the FOB, animals were randomized and the cage labels covered with the corresponding FOB number in order to make experimenters unaware of the animal's treatment group. Animals were observed in their home cage, during handling and in an open field. Observations were conducted over the functional domains of CNS activity, CNS excitation and sensorimotor, autonomic and physiological functions.

The checked (X) parameters were examined.

	HOME CAGE		HANDLING		OPEN FIELD
X	Posture*		Reactivity*	X	Mobility
X	Biting	X	Lacrimation* / chromodacryorrhea	X	Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*	X	Piloerection*	X	Convulsions
X	Abnormal movements*		Fur appearance	X	Tremors*
	Palpebral closure*		Palpebral closure*	X	Abnormal movements
X	Feces consistency	X	Respiratory rate+	X	Urination / defecation*
			Red/crusty deposits*		Grooming

	SENSORY	X	Mucous membranes /eye /skin colour	X	Gait abnormalities / posture*
	Approach response	X	Eye prominence*		Gait score*
	Touch response	X	Muscle tone*	X	Bizarre / stereotypic behaviour*
X	Startle response* (click response)	X	Skin cold to touch		Backing
X	Pain response*				Time to first step
X	Pupil response*				
X	Eye blink response		**PHYSIOLOGICAL**		**NEUROMUSCULAR**
	Forelimb extension	X	Body weight*		Hindlimb extensor strength
X	Hindlimb extension	X	Body temperature	X	Forelimb grip strength*
	Air righting reflex			X	Hindlimb grip strength*
	Olfactory orientation		**OTHER**	X	Hindlimb foot splay*
X	Hearing response (Preyer's reflex)	X	Abdomen appearance (distended/hard)		Rotarod performance
		X	Dehydration (skin fold disappears slowly)		

* Recommended for neurotoxicity studies based on Guideline OPPTS 870.6200, OECD 424

b. <u>Locomotor activity</u> - Locomotor activity (LMA) was assessed for all animals following the conduct of the FOB at the following intervals: once during acclimatization, on day 1 (at approximately 2 to 4 hours after dosing; time of peak effect), on days 7 and 14. An automated open field device (TRUSCAN, Coulbourn Instruments, Allentown / USA) was used to assess LMA. This device has been shown to detect increases as well as decreases in LMA. The test boxes (40 x 40 x 40 cm) are made of transparent plexiglass. Horizontal activity is monitored by 16 infrared beams per side that cross the box at 3 cm above the floor. To register vertical activity, a single row of 16 photobeams was mounted at an approximate height of ¾ of the rat's body length. LMA was recorded over 30 minutes, with data being collected at 3-minutes intervals. The data were collected using a Hewlett-Packard® PC, and the data files transferred onto the TOX CONTROL computer for storage. The test room had similar environmental conditions as the animal room (illuminated), with background noise provided by the fan of the PC. The following parameters were evaluated: Horizontal Activity: Total distance (in cm), Vertical Activity: Number of rearings (nos. of counts), Other Parameters: Time spent in central quadrant (center time, in sec.)

5. <u>Sacrifice and pathology</u>: Rats (five rats/sex/group) were subjected to deep anesthesia by intraperitoneal injection of pentobarbitone (about 400 mg/kg body weight) and euthanized by perfusion fixation *in situ* with 1 mL of 50 IU heparin followed by 0.1 M sodium phosphate buffered saline as the rinsing solution. A 10% formalin solution was used for fixation. On each sacrifice day, the order of sacrifice (perfusion fixation) was rotated through the treatment groups in a balanced design (e.g., group 1, group 2, group 3, group 4, group 1, group 2, etc.). The animals fixed by perfusion fixation were kept in a plastic container containing 4% formaldehyde for approximately 24 hours until post-fixation procedures were performed. Approximately 24 hours after perfusion fixation, the rats were necropsied and the visible organs or organ sections were assessed for gross abnormalities. At this time the appropriate nervous system tissues (as indicated below) were collected. The peripheral nerve tissues foreseen for plastic embedding were post-fixed with 4% paraformaldehyde, 5% glutaraldehyde in 0.1 M sodium phosphate buffered

saline (at pH 7.4) for approximately 2 hours at 4 ± 3 °C. Rinsing with 0.1 M sodium phosphate buffered saline was done 3 times for approximately 10 minutes each. Furthermore, the control and high dose groups were post-fixed with 2% osmium tetraoxide and 1.8% potassiumhexacyanoferrate II trihydrate (C6FeK4N6 • 3H2O) in 0.1 M sodium phosphate buffered saline for approximately 2 hours at room temperature (20 ± 5 °C).

The following tissues (X) were taken from all rats euthanized by perfusion fixation and preserved in the appropriate fixative and evaluated:

	CENTRAL NERVOUS SYSTEM		**PERIPHERAL NERVOUS SYSTEM**
	BRAIN		**SCIATIC NERVE**
X	Forebrain		Mid-thigh
	Centre of cerebrum	X	Sciatic notch*+
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons		Sural nerve
X	Medulla oblongata	X	Tibial nerve (proximal at knee & distal at lower leg)*+
	Upper brainstem		
X	Hippocampus		
	SPINAL CORD		Peroneal nerve
X	Cervical segment (C4-C7)	X	Lumbar dorsal root ganglion
X	Lumbar segment (L4-L5)	X	Lumbar dorsal root fibers
	Spinal nerve root-dorsal (cervical & lumbar) & ventral (cervical & lumbar)	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
X	Gasserian ganglion & nerve*+	X	Cervical dorsal root fibers
	Trigeminal nerves	X	Cervical ventral root fibers
X	Optic nerve*+		
X	Eyes*+		
X	Gastrocnemius muscle		

* Recommended for neurotoxicity studies based on Guideline OPPTS 870.6200, OECD 424

\+ Left component was taken and processed; right component was taken and stored in 4% formaldehyde solution.

All submitted tissues from control and high dose group animals were processed as follows. The low and mid dose group tissues were stored in buffer solution for possible future processing and examination.

Paraffin wax sections: The brain, spinal cord, eyes, Gasserian ganglia with nerve, and Gastrocnemius muscle from all (n = 5) perfused rats/sex/group were collected, as listed below.

Organ samples	Dose groups			
	1	2	3	4
Central nervous system:				
#Brain (coronal sections):		F*	F*	
- Frontal lobe	A			A
- Parietal lobe with diencephalon	A			A
- Midbrain with occipital and temporal lobe	A			A
- Pons	A			A
- Cerebellum	A			A
- Medulla oblongata	A			A
- Hippocampus	A			A
Eyes with retina and optical nerve	A	F	F	A
Spinal cord (cross and longitudinal sections):				
- Cervical cord (C4-C7)	A	F	F	A
- Lumbar cord (minimal L4-L5)	A	F	F	A
Peripheral nervous system:				
- Gasserian ganglia with nerve	A	F	F	A
- Gastrocnemius muscle	A	F	F	A
All gross lesions:	A	A	A	A

F: Preservation in 4% formaldehyde.
#, *: The brains from the group 2 and 3 rats were preserved *in toto*.
A: Paraffin embedding, sectioning and staining with hematoxylin-eosin (HE).

Plastic sections: The peripheral nerves from all (n = 5) perfused rats/sex/group were collected, as listed below.

Organ samples	Dose groups			
	1	2	3	4
Peripheral nervous system[1]:				
- Dorsal root ganglion (C4-C7)	T	P	P	T
- Dorsal root, longitudinal (C4-C7)	T	P	P	T
- Ventral root, longitudinal (C4-C7)	T	P	P	T
- Dorsal root ganglion (L4-L5)	T	P	P	T
- Dorsal root, longitudinal (L4-L5)	T	P	P	T
- Ventral root, longitudinal (L4-L5)	T	P	P	T
- Proximal sciatic nerve	T	P	P	T
- Proximal tibial nerve (at knee)	T	P	P	T
- Distal tibial nerve (at lower leg)	T	P	P	T

[1] When designed for processing, only the left component of each peripheral nerve was processed and examined, whereas the right component was collected and preserved in 4% formaldehyde solution for possible future processing/examination as deemed necessary by the study pathologist.
P: Storage of fixed specimen in buffer solution
T: Plastic embedding (epoxy resin), semithin sectioning and staining with 1% toluidine blue.

The various brain cross sections, spinal cord cross and longitudinal sections, eyes, Gasserian ganglia with nerve, and Gastrocnemius muscle from the group 1 and group 4 animals were embedded in paraffin, sectioned, stained with hematoxylin-eosin (H&E), and examined by light microscopy. These tissues from the group 2 and group 3 animals were preserved in buffered 4% formaldehyde solution for possible future embedding, sectioning, staining, and microscopic examination.

6. Positive controls - Acrylamide

II. RESULTS

A. Observations

1. Mortality: All animals survived their scheduled study period.

2. Clinical observations: No abnormalities were recorded during acclimatization in any dose groups. Treatment-related general daily cage side observations were limited to the 80 mg/kg males and 30 and 80 mg/kg females on days 1 and 2. On day 1 at approximately 1 hour post-dose, decreased activity was noted in 3/10 males at 80 mg/kg, 5/10 females at 30 mg/kg, and 10/10 females at 80 mg/kg. The severity of the decreased activity was minimum (grade 1) except for 7/10 females at 80 mg/kg in which the severity was moderate (grade 2). All 80 mg/kg females also exhibited swaying gait, abnormal gait, collapse, and ruffled fur. The severity of these findings was generally moderate (grade 2) except for ruffled fur (minimum) and collapse (no severity grade). On day 2, treatment-related clinical signs were limited to the 80 mg/kg females and consisted of a swaying gait in 4/10 females, decreased activity in 3/10 females, abnormal gait in

3/10 females, and ruffled fur in 5/10 females. In all cases the severity was minimal. On days 3 - 14, no treatment- related daily cage side observations were noted.

3. Detailed clinical observations: No abnormalities were recorded during acclimatization in any dose groups. On day 1 at approximately 5 hours post-dose, detailed clinical observations that were attributed to treatment (based on increased incidences and/or severities) were limited to the 80 mg/kg males and 30 and 80 mg/kg females. Detailed clinical observations noted at 5 hours post-dose on day 1 for the 80 mg/kg males that were attributed to treatment included decreased activity and soft feces. Although decreased activity (minimum) was also noted in one male each at 10 and 30 mg/kg (minimum), this finding could not be definitely attributed to treatment based on the low incidence, the absence of this finding in the 10 mg/kg females, and the presence of this finding in one control female (severity = grade 2). Detailed clinical observations noted post-dose on day 1 for the 80 mg/kg females that were attributed to treatment included reduced activity (moderate to marked), swaying gait (moderate), collapse, circling movement, paddling movements, muscle twitching, muscular hypotonus (moderate), hunched posture (minimum), ruffled fur (minimum to moderate), absence of push-reflex, absence of a pain response, and splayed hind limbs (marked). Detailed clinical observations noted post-dose on day 1 for the 30 mg/kg females that were attributed to treatment included reduced activity (minimum to moderate), swaying gait (minimum to moderate), collapse, muscle twitching, and ruffled fur (moderate). On days 7 and 14, there were no treatment-related detailed clinical observations. Although the iridic reflex was noted as absent in the left eye for one 30 mg/kg female on day 7, this finding is not attributed to treatment since this finding was not noted on days 1 and 14, nor was it noted at the higher dose of 80 mg/kg.

3. Body weight: Although not statistically significant, mean body weight gains over the day 1 - 8 interval were slightly lower for the 80 mg/kg males and the 30 and 80 mg/kg females (relative to controls), corresponding to the lower mean food consumption values noted over the day 1 - 2 interval for these groups. On Day 1, for males the body weights ranged from 205.8-209.0 g among groups and for females ranged from 147.9-150.3 g. Mean body weights at days 8 and 15 were generally similar for all groups. On Day 8, for males the body weights ranged from 228.6-235.1 g among groups and for females ranged from 158.8-163.7 g. Body weight gains for all groups (males or females) over the 2-week study duration (day 1 - 15) were similar. On Day 15, for males the body weights ranged from 260.1-264.5 g and for females ranged from 177.1-178.8 g.

Table IIA 5.7.1/02-3 Body Weights[a] (g)

BODY WEIGHTS (G) - SUMMARY
MALES

		Group 1 0 mg/kg	Group 2 10 mg/kg	Group 3 30 mg/kg	Group 4 80 mg/kg
ACCLIMATIZATION					
Day 1	MEAN	165.5	163.6 -	163.4 -	164.6 -
	ST.DEV.	5.3	4.7	7.1	4.8
	N	10	10	10	10
TREATMENT					
Day 1	MEAN	209.0	205.8 -	206.2 -	207.0 -
	ST.DEV.	7.0	7.0	10.1	6.6
	N	10	10	10	10
Day 8	MEAN	235.1	234.0 -	233.0 -	228.6 -
	ST.DEV.	8.8	6.2	11.8	8.8
	N	10	10	10	10
Day 15	MEAN	264.3	261.8 -	264.5 -	260.1 -
	ST.DEV.	9.9	11.5	16.8	12.7
	N	10	10	10	10

BODY WEIGHTS (G) - SUMMARY
FEMALES

		Group 1 0 mg/kg	Group 2 10 mg/kg	Group 3 30 mg/kg	Group 4 80 mg/kg
ACCLIMATIZATION					
Day 1	MEAN	122.5	122.6 -	122.5 -	123.4 -
	ST.DEV.	6.5	6.7	4.8	9.1
	N	10	10	10	10
TREATMENT					
Day 1	MEAN	147.9	150.3 -	148.1 -	149.7 -
	ST.DEV.	6.3	8.4	7.6	10.8
	N	10	10	10	10
Day 8	MEAN	163.7	161.7 -	158.8 -	160.6 -
	ST.DEV.	9.3	8.3	10.5	18.3
	N	10	10	10	10
Day 15	MEAN	178.8	177.8 -	177.1 -	178.3 -
	ST.DEV.	11.3	10.6	12.0	18.8
	N	10	10	10	10

a Data extracted from pages 127-128 in the study report

4. <u>Food consumption</u>: Mean food consumption on day 1 to 2 during acclimatization was slightly lower in males at 30 and 80 mg/kg and in females at 10 and 30 mg/kg, it was considered to be anomalous as these values are pre-treatment results. A treatment-related decrease in mean food consumption (relative to control) was noted for the 80 mg/kg males and the 30 and 80 mg/kg females over the day 1 - 2 assessment interval.

Table IIA 5.7.1/02-4 Mean food consumption values, expressed as g/rat/day and (percent change from control) on days 1 - 2

	Group 1 0 mg/kg	Group 2 10 mg/kg	Group 3 30 mg/kg	Group 4 80 mg/kg
Males	18.51	19.81 (+7.0)	17.83 (- 3.7)	**13.92 (- 24.8)****
Females	14.67	14.19 (- 3.3)	**9.26 (- 36.9)****	**4.59 (- 68.7)****

** $p \leq 0.01$

Data extracted from pages 40 of the study report

5. <u>Neurobehavioural assessments</u>:

a) Functional observational battery (FOB): During acclimatization no abnormalities in any male or female dose groups were detected during the FOB evaluation. Treatment-related FOB findings (based on increased incidences and/or severities) were limited to the 80 mg/kg males and 30 and 80 mg/kg females. FOB findings noted at 2 to 4 hours post-dose on day 1 for the 80 mg/kg males that were attributed to treatment included reduced activity and soft feces. Although reduced activity also noted in one male each at 10 and 30 mg/kg, this finding could not be definitively attributed to treatment based on the low incidence, the absence of this finding in the 10 mg/kg females, the presence of this finding in one control female, and the lack of any correlating findings in the 10 and 30 mg/kg males. The finding of reduced activity in the FOB for the 80 mg/kg males correlates to the higher incidence of decreased activity noted for this group at the day 1 post-dose daily cage side clinical signs. FOB findings noted post-dose on day 1 for the 80 mg/kg females that were attributed to treatment included reduced activity, abnormal gait, paralysis, circling movement, paddling movements, reduced muscle tone, and piloerection. FOB findings noted post-dose on day 1 for the 30 mg/kg females that were attributed to treatment included reduced activity, abnormal gait, and piloerection. Although tremor was noted in one female at 30 mg/kg and 80 mg/kg, this finding could not be definitively attributed to treatment based on the presence of this finding in one control female and the lack of a dose-response relationship. On days 7 and 14, no treatment related findings were observed during the FOB assessments. The only finding recorded on day 7 was the impaired papillary reflex in the left eye of one 30 mg/kg female which was considered incidental to treatment, given the low incidence of this finding, the absence of any dose-response relationship, and the absence of this finding on days 1 and 14.

Table IIA 5.7.1/02-5. Selected FOB parameters at Day 1 post-dose[a]

Finding	Control		10 mg/kg		30 mg/kg		80 mg/kg	
(maximum severity score)	Males	Females	Males	Females	Males	Females	Males	Females
Soft Feces (2)	0	0	0	0	0	0	1 (1.0)	0
Reduced Activity (2)	0	1 (2.0)	1 (1.0)	0	1 (1.0)	6 (1.7)	2 (1.0)	7 (2.0)
Muscle Tone Reduced (2)	0	0	0	0	0	0	0	1 (2.0)
Tremor (2)	0	0	0	0	0	1 (1.0)	0	1 (1.0)
Hunched posture (1)	0	0	0	0	0	0	0	1 (1.0)
Circling move't (2)	0	0	0	0	0	0	0	1 (1.0)
Abnormal Gait (2)	0	0	0	0	0	4 (1.0)	0	4 (1.0)
Paddling move't (1)	0	0	0	0	0	0	0	1 (1.0)
Paralysis (2)	0	0	0	0	0	0	0	2 (1.0)
Piloerection (2)	0	0	0	0	0	2 (2.0)	0	7 (1.6)
Recumbency (1)	0	1 (1.0)	0	0	0	0	0	0

[a] Data obtained from page 37 in the study report

b) FOB Body Temperature: The significantly lower mean body temperatures measured at 2 - 4 hours post-dose on day 1 in males at 80 mg/kg and females at 30 and 80 mg/kg are attributed to the test article. No treatment-related effects on body temperature were noted on days 7 and 14.

Table IIA 5.7.1/02-7. Rectal body temperature (°C; mean, % change in parenthesis) at Day 1 post-dose[a]

	Group 1 0 mg/kg	Group 2 10 mg/kg	Group 3 30 mg/kg	Group 4 80 mg/kg
Males	38.0	38.0 (+0.0%)	38.1 (+0.3%)	**37.7 (-0.8%)***
Females	37.9	38.0 (+0.3%)	**35.1 (-7.4%)****	**34.4 (-9.2%)****

* $p \leq 0.05$ ** $p \leq 0.01$

[a] Data obtained from pages 38 in the study report

c) FOB grip strength: Treatment-related decreases in group mean grip strength were limited to the 30 and 80 mg/kg females at 2 - 4 hours post-dose. On day 1, forelimb grip strength values were statistically significantly reduced at 30 and 80 mg/kg females, and hind limb grip strength values were statistically significantly reduced for the 80 mg/kg females. Grip strength values in males were not affected by the test article. There were no treatment-related effects on fore- and/or hind limb grip strength values on days 7 and 14.

Table IIA 5.7.1/02-8. Mean fore- and hind limb grip strength, expressed as kg and (percent change from control), at 2 - 4 hours post-dose

	Group 1 0 mg/kg	Group 2 10 mg/kg	Group 3 30 mg/kg	Group 4 80 mg/kg
Forelimb males	0.57	0.63 (+10.5%)	0.61 (+7.0%)	0.64 (+12.3%)
females	0.60	0.61 (+1.6%)	**0.44 (-26.7%)***	**0.34 (-43.3%) ** **
Hindlimb males	0.31	0.32 (+3.2%)	0.33 (+6.5%)	0.33 (+6.5%)
females	0.28	0.25 (- 10.7%)	0.24 (- 14.3%)	**0.21 (-25.0%)***

* $p \leq 0.05$ ** $p \leq 0.01$

[a] Data obtained from pages 38 in the study report

d) FOB landing foot splay: Landing foot splay was not affected by treatment with the test article.

e) Locomotor activity: Treatment-related locomotor findings were limited to the 30 and 80 mg/kg females at approximately 2 - 4 hours post-dose on day 1, as shown in text table 4 below. Total distance and rearing activity were significantly lower in females at 30 and/or 80 mg/kg, and total center time was significantly increased in females at 80 mg/kg. There were no statistically significant alterations in any of these three locomotor activity parameters for males at approximately 2 - 4 hours post-dose. There were no treatment-related changes in any of the locomotor activity parameters for either males or females on days 7 and 14. The isolated statistically significant differences from control occasionally noted for the 10 mg/kg males (day 1/distance/bin7 and day 7/center time/bin6, 10 mg/kg females (acclimatization/distance/bin6 and acclimatization/center time/bin6), 30 mg/kg females (day 7/distance/bin4and day 14/rearing/bin2), 80 mg/kg males (day 1/number of rearings/bin9) and 80 mg/kg females (day 7/distance/bin6) are all considered to be incidental to treatment.

Table IIA 5.7.1/02-9. Mean locomotor activity parameters obtained over a 30 minute session at 2 - 4 hours post-dose

	Control		10 mg/kg		30 mg/kg		80 mg/kg	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Total Distance (cm)	1635.0	2611.2	2140.0	2323.2	1711.7	**832.7****	1451.7	**778.6****
% Change from Control	---	---	+30.9	-11.0	+4.7	**-68.1**	-11.2	**-70.2**
Total No. of Rearings (counts)	29	55	37	48	21	**14****	17	**8****
% Change from Control	---	---	+27.6	-12.7	-27.6	**-74.5**	-41.4	**-85.5**
Total Center Time (sec)	165	184	176	114	97	44	68	**770****
% Change from Control	---	---	+6.7	-38.0	-41.2	-76.1	-58.8	**+318.5**

**p ≤0.01

a Data extracted from pages 39 of the study report

6. Sacrifice and pathology:

a) **Organ weights:** No treatment-related effects on absolute, relative and adjusted brain weights were evident in males or females.

b) **Macroscopical findings:** There were no gross lesions recorded at necropsy.

c) **Microscopical findings:** There were no neurohistopathological lesions present that could be attributed to treatment with the test item. One 80 mg/kg male was noted with single nerve fiber degeneration (minimal severity) in the proximal tibial nerve, and another 80 mg/kg male was noted with single nerve fiber degeneration in the dorsal root ganglion nerve (minimal severity). Both of these findings were within the range of normal background lesions which may be recorded in animals of this strain and age (Eisenbrandt et al., 1990), and are not attributed to treatment.

III Discussion

A. Investigator's conclusions (extracted from page 42-43 in the study report):

Following the administration of a single oral gavage dose of SYN545192 to Wistar rats at 10, 30 and 80 mg/kg, evidence of toxicity was limited to the 80 mg/kg males and the 30 and 80 mg/kg females. The majority of treatment-related findings occurred post-dose on

day 1, and included multiple clinical observations, decreases in mean body temperature, decreases in locomotor activity parameters, and/or decreases in mean grip strength. Other findings noted for the 80 mg/kg males and the 30 and 80 mg/kg females included lower mean food consumption over the first day of treatment (day 1 - 2) with corresponding reductions (slight but not statistically significant) in body weight gains over the first week of treatment (days 1 - 8). All of these treatment-related changes were transient. Based on these findings, the no observed- adverse-effect level (NOAEL) for systemic toxicity was 10 mg/kg for females and 30 mg/kg for males.

No clinical and/or behavioral signs of toxicity were evident in any of the rats after day 2, and mean overall (day 1 - 14) food consumption and body weight gain values were not affected by the treatment. At study termination no treatment-related neurohistopathological lesions were present. Thus the transient treatment-related findings noted in the 80 mg/kg males and the 30 and 80 mg/kg females were attributed to the generalized systemic toxicity rather than to specific neurotoxic effects.
Based on the results of this study, the NOAEL for neurotoxicity was 80 mg/kg for both males and females.

B. Reviewer's conclusions:

The Agency concurs with study author's conclusions and selection of NOAEL and LOAEL. The clinical signs, changes in motor activity and grip strength could be due to the general toxicity via the inhibition of mitochondrial function, the pesticidal mode of action of the test article. Females were the more sensitive gender in multiple measurements.

C. Deficiencies:

In some sections, the observations were noted at 5 hours post-dosing, however, the FOB measurements were generally made at 2-4 hours after dosing. Only the control compound data for histopathology was presented (i.e. acrylamide) and that study was somewhat dated, performed in 2005.

D. References:

Sommer E W, Flade D and Krinke G J (2005): Acrylamide: 28-Day Oral Neurotoxicity (Gavage) Validation Study in Rats. RCC Study Number 852323, p 1-375.

Report:	IIA 5.7.4/02 Sommer E (2011). **SYN545192 - 13 Week Dietary Neurotoxicity Study in Rats.** Harlan Laboratories Ltd., Zelgliweg 1, CH-4452 Itingen / Switzerland. Laboratory Report No. C96067. Issue date 11 Nov 2011. Unpublished. MRID # 48604457

Guidelines: 90-Day Dietary Neurotoxicity Study. OECD 424 (1997): US EPA OPPTS 870.6200 (1998): EU Council Directive 67/548//EEC B.43 (2000): JMAFF Notification No. 12 NohSan 8147 (2000)

Sponsor: Syngenta Crop Protection, LLC, 410 Swing Road Post Office Box 18300 Greensboro, NC 27419-8300 USA

Executive Summary: In this sub-chronic dietary neurotoxicity study (MRID # 48604457), groups of twelve male and twelve female RccHan:Wistar (SPF) rats were fed SYN545192 admixed to their diet for 13 weeks at concentrations of 0, 100, 400, and 800 ppm (males: 0, 6.31, 25.95, or 50.67 mg/kg/day) or 0, 100, 250, and 500 ppm (females: 0, 7.48, 19.17, or 37.99 mg/kg/day). General cage side observations were made for all animals prior to study start and daily throughout the study. Detailed clinical observations comprising open field evaluation of clinical signs were performed in a randomized order once prior to initiation of treatment and once weekly thereafter. Functional observation batteries (FOBs) including quantitative assessments of landing foot splay, sensory perception and muscle weakness were performed for all animals in a randomized order once prior to initiation of treatment, and in weeks 2, 5, 9 and 13. Locomotor activities were assessed after each FOB evaluation. Food consumption values were recorded weekly throughout the study. Body weights were recorded once prior to initiation of treatment on day 1, and weekly thereafter. Ophthalmoscopic examinations were performed for all animals prior to initiation of treatment on day 2, and for the control and high-dose animals during week 13. At the end of the treatment period, 5 rats/sex/group were perfusion-fixed *in situ.* The brain weights were measured and selected nervous tissues were dissected, processed and examined microscopically.

Dietary administration of SYN545192 to Wistar rats for 13 weeks at concentrations of 0, 100, 400 and 800 ppm (males) or 0, 100, 250 and 500 ppm (females) resulted in treatment related decreases in body weight gain and/or food consumption for the 400 and 800 ppm males and the 500 ppm females. These decreases in body weight gain and food consumption are attributed to generalized system effects rather than specific neurotoxic effects. The ultimate decrease in body weights were less than 10% in both genders, thus not considered to be adverse. The decreased food consumption and decreased body weight gains did not have a functional adverse impact on the ultimate body weights, thus were not considered adverse.

The NOAEL values of the study are the highest doses tested, or 50.67 mg/kg/day in males and 37.99 mg/kg/day in females and the LOAEL values were not determined. There was no evidence of neurotoxicity based on the absence of treatment-related clinical

observations, ophthalmoscopic findings, FOB findings, locomotor activity changes, and/or neuropathologic findings (i.e., brain weights, macroscopic findings, and microscopic findings). **Thus, the NOAEL for neurotoxicity is 800 ppm for males and 500 ppm for females, corresponding to 50.67 mg/kg body weight/day in males and 37.99 mg/kg body weight/day in females.**

The study is considered to be acceptable/non-guideline due to the lack of control compound data.

Compliance: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No claim of CBI was made for any information contained in this document.

I. MATERIALS AND METHODS

A. Materials:

Test Material:	SYN545192
Description:	beige powder
Lot/Batch number:	SMU9BP005
Purity:	SYN545192, 97.0%
CAS #:	NA
Stability of test compound:	Stable under storage conditions of At room temperature (20 ± 5 °C), in the original container, away from direct sunlight.. Expiry date 28 Feb 2013
Vehicle:	Microgranulated standard Kliba Nafag 3433 rodent maintenance diet,
Positive control:	NA

Test Animals:

Species	Rat
Strain	HanRcc: WIST (SPF)
Age at dosing	7 weeks
Weight at dosing	Males 113.1-142.6 g, females 88.6-125.9 g.
Source	Harlan Laboratories, B.V. Kreuzelweg 53 5961 NM Horst / Netherland
Housing	The animals were gang-housed with 3 animals per cage (i.e., 4 cages/group/sex) using Makrolon type-4 cages with wire mesh tops and standard soft wood bedding
Acclimatization period	7 days for males, 8 days for females.
Diet	Microgranulated standard Kliba Nafag 3433 rodent maintenance diet, batch nos. 63/10 until 17 Feb 2011; 02/11 from 17 Feb 2011 (Provimi Kliba AG, 4303 Kaiseraugst, Switzerland) *ad libitum*
Water	Community tap-water from Itingen was available *ad libitum*
Environmental conditions	Temperature:22±3°C Humidity: 30-70% Air changes: 10-15 changes/hour Photoperiod: 12 hours light/12 hours dark

B. Study Design and Methods:

1. In-life dates: Start: 06-Dec 2010 (males), 07-Dec 2010 (females) End: 10-March 2011 (males), 11- March 2011(females)

2. Animal assignment and treatment: Animals were assigned to the test groups noted in Table IIA 5.7.4/02-1 by a computer-generated random algorithm program. Animals were fed dietary admixtures, freshly prepared every week for a total of 13 weeks.

At study termination, 5 rats/sex/group were perfused *in situ*. Selected nervous system tissues were removed, and preserved in an appropriate fixative. From the five animals/sex/group sacrificed by perfusion fixation, the brain was removed and the weight recorded. The remaining animals were sacrificed and discarded. Submitted tissues from high dose and control animals were examined by light microscopy.

Table IIA 5.7.4/02-1. Study design

Experimental Parameter	Study Design			
	Control	Low Dose	Mid Dose	High Dose
Total number of Animals/sex/group	12/sex	12/sex	12/sex	12/sex
Behavioral Testing (FOB, Motor Activity)	12/sex	12/sex	12/sex	12/sex
Neuropathology	5/sex	5/sex	5/sex	5/sex

Males: 0, 6.31, 25.95, or 50.67 mg/kg/day, Females: 0, 7.48, 19.17, or 37.99 mg/kg/day

3. Dose level selection (extracted from p. 24 in the study report):

Dose levels for this subchronic neurotoxicity study were based on several toxicity studies in rats, including a 14-day study, a 28-day study, a 90-day study, on ongoing 2-year study, and an ongoing reproductive toxicity study. All these studies demonstrated that a decrease in body weight gain was a consistent indicator of SYN545192 toxicity, with females being more sensitive than males. Using dose levels based on the findings from previous studies, a preliminary 28-day dietary neurotoxicity study was performed (Harlan Study C96056) in which Wistar (RccHanTM:WIST) rats administered SYN545192 in their diets at 0 (control), 100, 500, 1000 ppm (males) or 750 ppm (females) for 28 consecutive days exhibited no mortality, no treatment-related clinical signs, nor any findings attributed to neurotoxicity. Findings that were attributed to treatment were limited to decreased body weight gain (500 ppm females and 750 ppm females), and decreased food consumption (1000 ppm males, and 500 and 750 ppm females). However, the body weight data in this study were suspect based on the growth pattern of the control rats, particularly for male rats. Therefore the body weight results for other rat studies with SYN545192 were also used in determining dose levels for the subchronic neurotoxicity study. Dose levels for males were based on the results of the robust database from previous and ongoing rat studies. Based on this database, a dietary concentration of 800 ppm was anticipated to result in a lower mean body weight of approximately 10% (relative to controls) after 13 weeks of treatment. A marginally lower mean body weight was anticipated at 400 ppm, and the NOAEL was anticipated to be 100 ppm. Dose levels for females were based on the results of the preliminary 28-day neurotoxicity study and the robust database from previous and ongoing studies. A dietary concentration of 500 ppm was anticipated to result in a lower mean body weight of approximately 10% (relative to controls) after 13 weeks of treatment. A marginally lower mean body weight was anticipated at 250 ppm, and the NOAEL was anticipated to be 100 ppm.

4. <u>Test substance preparation and analysis</u>: Dietary admixtures were prepared using basal diet and the test item as supplied. No correction factor was applied during the preparation of the dietary admixtures. Fresh batches of the feed admixtures for this study were prepared weekly for week 1 and once every 3 weeks from treatment week 2 to the end of the study. SYN545192 was weighed into a tared glass baker on a suitable precision balance, and mixed with micro-granulated feed separately for each dose group. The feed preparations were identified on the bags by declaration of group/sex and bar code. Control feed for the animals of group 1 was prepared similarly, but without test item.

Concentration and homogeneity of the test item in the diets were determined in samples taken from the diet preparations made on Dec. 6, 8 and 14, 2010 as well as Jan. 3 and 26, 2011. For assessment of concentration and homogeneity, samples were collected from the top, middle and bottom of every dietary admixture (including controls). Representative diet samples were dispatched to the analytical laboratories internally (under ambient conditions) and either directly analyzed, or stored frozen at -20 ± 5 °C until analysis. The analytical method was provided by the Sponsor and validated within Harlan Laboratories study C94043 / Syngenta Task no. TK0002619.

Results –

Homogeneity Analysis: The coefficients of variation calculated for top, middle, and bottom samples of each preparation were found to be ≤ 5% with only seven exceptions which slightly exceeded the criterion [14-Dec-10 preparation for group 4-female (6.1%); 26-Jan-11 preparations for group 2-males (5.4%), group 3-males (5.4%), group 3-females (10.5%), and group 4-males (8.5%); and 14-Feb-11 preparations for group 2-males (5.1%) and group 2-females (6.0%)].

Stability Analysis: Diet preparations prepared at 20, 720 and 1500 ppm were determined to be stable when stored for 28 days at room temperature (20 ± 5 °C) under ambient light conditions and when stored frozen (-20 ± 5 °C) for 21 days (Harlan Laboratories study C94043).

Concentration Analysis: The SYN545192 concentrations in 97 out of a total of 99 diet samples were determined to meet the protocol-specified criterion (within ±10% of target) with the exception of two feed samples that deviated only slightly from this criterion [26-Jan-11 preparations for group 2-males middle sample (87.9% of target) and group 3-females bottom sample (84.5% of target)].

As the great majority of samples met the target criterion, and since the results from those analyses that did not meet the criterion deviated only slightly from target, the analytical results confirmed that the diet preparations used on study were properly prepared and appropriate for interpretation of the study findings.

5. Statistics: The following statistical methods were used to analyze the food consumption, body weight and cumulative body weight gain, body temperature, landing foot splay, locomotor activity, organ weights, adjusted organ weights, and grip strength:

• A one-way analysis of variance (ANOVA) was applied and
pairwise comparisons were performed against the control group (group 1), using the Dunnett's test . For analysis of adjusted organ weights, the
Dunnett analysis of covariance with the body weight as covariate was applied. The Fisher's exact test was applied for ophthalmoscopy and
histopathological findings.
• The Steel-test (many-one rank test) was applied instead of the
Dunnett's-test when the data cannot be assumed to follow a normal distribution.

C. Methods

1. Mortality and clinical observations: Observations for viability / mortality were recorded once daily. All animals were observed for clinical signs once prior to onset of treatment; and once daily during the treatment period. The animals were monitored for detailed clinical observations in their home cages, outside their home cages in a standard arena and in the hand. These observations were performed in random sequence once before commencement of administration and once weekly thereafter. All animals were evaluated using a FOB at acclimatization (prior to initiation of treatment) and at weeks 2,

5, 9 and 13. Evaluations included quantitative assessments of landing foot splay, sensory perception and muscle weakness. The FOB was conducted on all animals which did not otherwise reveal signs of toxicity to an extent that would have significantly interfered with the functional test performance. For the conduct of the FOB, animals were randomized and the cage labels covered with the corresponding FOB number in order to make experimenters unaware of the animal's treatment group. Animals were observed in their home cage, during handling and in an open field. Observations were conducted over the functional domains of CNS activity, CNS excitation and sensorimotor, autonomic and physiological functions.

2. Body weights: Body weights were recorded in all animals at least once during the acclimatization period, on the day of initiation of dietary treatment (day 1), and weekly thereafter.

3. Food consumption: The food consumption was monitored weekly throughout the study and reported weekly during week 1 to 13. During the measurement of the Week 13 food consumption, no animals were food deprived (i.e., fasted).

4. Ophthalmoscopy: Ophthalmoscopic examinations were performed in all animals during acclimatization on day 2. At week 13, animals of groups 1 and 4 were examined. As there were no test item-related findings in group 4 animals, animals of the low and mid dose groups were not examined during week 13.

5. Neurobehavioral Assessment:

a) Functional observational battery (FOB): All animals were evaluated using a FOB at acclimatization (prior to initiation of treatment) and at weeks 2, 5, 9 and 13. Evaluations included quantitative assessments of landing foot splay, sensory perception and muscle weakness. The FOB was conducted on all animals which did not otherwise reveal signs of toxicity to an extent that would have significantly interfered with the functional test performance. For the conduct of the FOB, animals were randomized and the cage labels covered with the corresponding FOB number in order to make experimenters unaware of the animal's treatment group. Animals were observed in their home cage, during handling and in an open field. Observations were conducted over the functional domains of CNS activity, CNS excitation and sensorimotor, autonomic and physiological functions.

The CHECKED (X) parameters were examined.

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture*		Reactivity*		Mobility
	Biting	X	Lacrimation* / chromodacryorrhea		Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*	X	Piloerection*		Convulsions
X	Abnormal Movements*		Fur appearance	X	Tremors*
	Palpebral closure*		Palpebral closure*		Abnormal movements
	Feces consistency	X	Respiratory rate+	X	Urination / defecation*

			Red/crusty deposits*	X	Grooming
	SENSORY OBSERVATIONS		Mucous membranes /eye /skin colour		Gait abnormalities / posture*
	Approach response+	X	Eye prominence*		Gait score*
	Touch response+	X	Muscle tone*		Bizarre / stereotypic behaviour*
X	Startle response*	X	Recumbency		Backing
X	Pain response*	X	Vocalization		Time to first step
X	Pupil response*		Abdomen appearance		
X	Eye blink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension	X	Body weight*		Hindlimb extensor strength
X	Hindlimb extension	X	Body temperature+	X	Forelimb grip strength*
	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation			X	Hindlimb foot splay*
	Vision response		**OTHER OBSERVATIONS**		Rotarod performance
	Audition response	X	Eye appearance (opaque, discoloured, reddened)		
	Righting response				
	Visual placing				

*Required parameters; +Recommended parameters

b) <u>Locomotor activity</u>: Locomotor activity (LMA) was assessed after conduct of the FOB at acclimatization (prior to initiation of treatment) and at weeks 2, 5, 9 and 13. LMA was assessed using an automated open field device (TRUSCAN, Coulbourn Instruments, Allentown / USA). This device has been shown to detect increases as well as decreases in locomotor activity. The test boxes (40 x 40 x 40 cm) are made of transparent plexiglass. Horizontal activity is monitored by 16 infrared beams per side that cross the box at 3 cm above the floor. To register vertical activity, a single row of 16 photobeams is mounted at an approximate height of ¾ of the rat's body length. LMA was recorded over 30 minutes and stored on a HP-PC at 3-minute intervals (bins). Activity measurements were performed between 8:00 AM and 3:00 PM. The test room had essentially the same environmental conditions as the animal room (illuminated), with background noise provided by the fan of the PC. Animals were allocated to the different runs and test boxes by means of a Latin square design so that treatment groups were balanced across test boxes and time. Males and females were tested on separate days.

The following parameters were evaluated:
Horizontal activity: Total distance (in cm)
Vertical activity: Number of rearings (counts)
Other parameters: Time spent in central quadrant (center time, in sec.)

6. <u>Sacrifice and pathology</u>: The first 5 rats/sex/group of the surviving animals were subjected to deep anaesthesia by intraperitoneal injection of Esconarkon® (about 200 mg/kg body weight, Streuli Pharma SA, Uznach, Switzerland) and sacrificed by perfusion fixation *in situ* with 1 mL of 50 IU heparin followed by 0.9% saline buffer as the rinsing solution. For fixation a 4% formaldehyde solution was used. After perfusion fixation, the brain with the optic nerve was removed and weighed. All organs and tissues that were not needed for further processing were removed and discarded. The animals

fixed by perfusion fixation were kept *in situ* in a plastic container containing 4% formaldehyde for approximately 24 hours until post-fixation was performed. The peripheral nerve tissues foreseen for plastic embedding were post-fixed with 4% paraformaldehyde, 5% glutaraldehyde in 0.1 M sodium phosphate buffered saline (at pH 7.4±0.2) for approximately 2 hours at 5 ± 3 °C. Rinsing with 0.1 M sodium phosphate buffered saline was done 3 times for approximately 10 minutes each. Furthermore, the control and high dose groups were post-fixed with 2% osmium tetraoxide and 1.8% potassiumhexacyanoferrate II trihydrate ($C_6FeK_4N_6 \cdot 3\ H2O$) in 0.1 M sodium phosphate buffer for approximately 2 hours at room temperature (20 ± 5 °C) and afterwards briefly rinsed once with 0.1 M sodium phosphate buffered saline. The animals fixed by perfusion were necropsied, and the visible organs or organ sections were assessed for gross abnormalities. At scheduled termination after 13 weeks of treatment, the remaining animals were anesthetized with isoflurane and terminated by decapitation without further examinations (i.e., necropsies were not performed).

	CENTRAL NERVOUS SYSTEM		**PERIPHERAL NERVOUS SYSTEM**
	BRAIN		**SCIATIC NERVE**
X	Forebrain		Mid-thigh
X	Center of cerebrum	X	Proximal, below sciatic notch*
X	Midbrain (including hippocampus)		
X	Cerebellum		**OTHER**
	Pons		Sural Nerve
	Upper brainstem	X	Tibial Nerve (proximal and distal)*
	Medulla oblongata		Peroneal Nerve
	SPINAL CORD	X	Lumbar dorsal root ganglion (L4-L5)*
X	Cervical swelling (C4-C7)		Lumbar dorsal root fibers (L4-L5)
X	Lumbar swelling (L4-L5)	X	Lumbar ventral root fibers (L4-L5)*
	Thoracic swelling	X	Cervical dorsal root ganglion (C4-C7)*
	OTHER		Cervical dorsal root fibers (C4-C7)
X	Gasserian Ganglion	X	Cervical ventral root fibers (C4-C7)*
	Trigeminal nerves	X	Spinal dorsal root ganglion
X	Optic nerve		
X	Eyes		
X	Gastrocnemius muscle		
X	Gross lesions		

* = left component was taken and processed, right component was taken and stored in 4% formaldehyde solution.

The brain (with the optic nerve) weight was determined after fixation from rats euthanized by perfusion fixation. For determination of the relative brain weights, the terminal body weights were used.

The following organs/tissues were preserved in neutrally buffered 4% formaldehyde solution:
1. Brain (remaining material after trimming)
2. Spinal cord (sections from cervical and lumbar cord)
3. All gross lesions

The tissue specimens for epoxide embedding (as presented in the table below) from 5 subset B rats/sex in the control and high-dose groups were removed after post fixation, embedded in plastic (epoxy resin), sectioned, stained with toluidine blue, and examined by light microscopy. Tissues from the mid and low dose groups were also processed.

Tissue Samples	Dose Groups			
	1	2	3	4
Peripheral Nervous System[1]:				
Dorsal root ganglion, (min. 2 of C4–C7) (L)	T5	T5	T5	T5
Dorsal root, longitudinal (min. 2 of C4-C7) (L)	T5	T5	T5	T5
Ventral root, longitudinal (min. 2 of C4-C7)(L)	T5	T5	T5	T5
Dorsal root ganglion, (min. 2 of L4-L5) (L)	T5	T5	T5	T5
Dorsal root, longitudinal (min. 2 of L4-L5) (L)	T5	T5	T5	T5
Ventral root, longitudinal (min. 1 of L4-L5) (L)	T5	T5	T5	T5
Proximal sciatic nerve below sciatic notch (T)	T5	T5	T5	T5
Proximal tibial nerve (at knee) (T)	T5	T5	T5	T5
Distal tibial nerve (at lower leg) (T)	T5	T5	T5	T5

T: Plastic embedding (epoxy resin), semithin sectioning and staining with 1% toluidine blue
5: All perfused animals per group and sex
(T), (L): Transverse, longitudinal sections

These epoxy resin sections were examined by light microscopy and assessed.

The tissue specimens for paraffin embedding (as presented in the table below) from 5 subset B rats/sex in the control and high-dose groups were removed after post fixation, embedded in plastic (epoxy resin), sectioned, stained with hematoxylin-eosin (HE), and examined by light microscopy. Tissues from the mid and low dose groups were also processed and examined.

Tissue Samples	Dose Groups			
	1	2	3	4
Central Nervous System:				
Brain (cross sections):		F5*	F5*	
Frontal lobe	A5			A5
Parietal lobe with diencephalon	A5			A5
Midbrain (hippocampus) with occipital and temporal lobe	A5			A5
Pons	A5			A5
Cerebellum	A5			A5
Medulla oblongata	A5			A5
Eyes with retina and optical nerve (L)	A5	F5*	F5*	A5
Spinal cord (cross and longitudinal sections):				
Cervical cord (C4–C7) (T,L)	A5	A5	A5	A5
Lumbar cord (min. L4–L5) (T,L)	A5	A5	A5	A5
Peripheral nervous system:				
Gasserian ganglia with nerve (L)	A5	A5	A5	A5
Gastrocnemius muscle (T,L)	A5	A5	A5	A5
Proximal sciatic nerve below sciatic notch (L)	A5	A5	A5	A5
Proximal tibial nerve (at knee) (L)	A5	A5	A5	A5
Distal tibial nerve (at lower leg) (L)	A5	A5	A5	A5
All gross lesions:	A2	A2	A2	A2

A: Paraffin embedding, sectioning and staining with hematoxylin-eosin (HE)
5: All perfused animals per group and sex
* In toto.
2: All animals affected from the group fixed by perfusion.
(T), (L): Transverse, longitudinal sections
F: Preservation in neutrally buffered 4% formaldehyde solution for possible further processing, in the case that effects were recorded in tissues of the high dose group.

These HE-stained paraffin sections were examined by light microscopy and assessed. As they were a few neuropathological findings recorded in the high dose groups, the intermediate dose groups were processed histotechnically and examined by light microscopy.

7. <u>Positive Controls</u>: NA

II. RESULTS

A. Observations

1. Mortality: There was no unscheduled mortality noted in this study. All animals survived the scheduled study period.

2. Clinical signs: No treatment-related general cage side observations were observed during the study period. The only remarkable findings were hair loss and wound at the right shoulder of control male no. 7. These findings were considered incidental to treatment since they occurred with low incidence and frequency, were noted in a control rat, and represent typical background findings in rats of this strain and age. No treatment-related detailed clinical observations were present during the weekly assessments.
The only remarkable findings were scabs at the right shoulder in control male no. 7 and absent iridic reflex – left eye in mid-dose female no. 82 during week 2. These findings were considered incidental to treatment since they occurred with low incidence and frequency, lacked a dose-response relationship, and represent typical background findings in rats of this strain and age.

3. Ophthalmoscopy: Ophthalmoscopic examinations revealed no test item-related effects. Corneal opacity, persistent pupil membrane in the lens and persistent hyaloid vessel and vitreous floaters in the vitreous body were noted in individual animals of the control or high dose groups. These observations represent typical background findings in rats of this strain and age, and therefore are not attributed to treatment.

4. Body weight and body weight gain: Statistically significant decreases in mean overall (day 1 - 92) body weight gains were noted for the 400 and 800 ppm males and the 500 ppm females, relative to controls, and are attributed to treatment. Similarly, the statistically significant lower mean body weight after 13 weeks of treatment (day 92), relative to controls, noted for these treatment groups are attributed to treatment. While the decreases in body weight gains were over 10% for both genders, however, the functional decreases in the ultimate body weights were less than 10%, thus not considered to be adverse for either gender. The decreases for the 800 ppm males and 500 ppm females are consistent with the decreased food consumption noted for these groups. The slight, but not statistically significant, decreases in mean body weight and overall body weight gain noted for the 250 ppm females could not be conclusively attributed to treatment.

Table IIA 5.7.4/02–2: Mean body weights and body weight gains, expressed as g (and percent changes from control) after 13 weeks of treatment.

	Group 1	Group 2	Group 3	Group 4
MALES	**0 ppm**	**100 ppm**	**400 ppm**	**800 ppm**
Mean body weight gain (day 1 – 92)	275.3	272.3 (- 1.1%)	240.2* (- 12.7%)	234.5** (- 14.9%)
Mean body weights (day 92)	446.8	444.9 (- 0.4%)	409.8* (- 8.3%)	412.1* (- 7.8%)
FEMALES	**0 ppm**	**100 ppm**	**250 ppm**	**500 ppm**
Mean body weight gain (day 1 – 92)	121.3	124.4 (+2.6%)	110.1 (-9.2%)	93.1** (- 23.2%)
Mean body weights (day 92)	256.8	259.1 (+0.9%)	248.4 (-3.3%)	231.7** (- 9.8%)

*/** $p \leq 0.05 / p \leq 0.01$

Data taken from page 37 in the study report

5. <u>Food consumption</u>: Statistically significant decreases in the food consumption values, relative to control values, were noted for the 400 and 800 ppm males (days 82-83 at -12.1% and -14.5%, respectively) as well as the 500 ppm females (days 47-48 at -18.4% & days 75-76 at -22.6%). These decreases correlated with the decreased mean overall body weight gains noted in these treatment groups. Since slightly lower food consumption values were noted at most of the weekly intervals (which were infrequently statistically significantly lower), the slightly lower mean overall food consumptions observed for these treatment groups are attributed to treatment. There was a considerable variability in the food consumption in both genders with time. In males, the period before days 82-83 had a decreased food consumption of only -0.4% and an increased consumption of +6.0% during the following period. In females, in the period before days 47-48, the decrease in food consumption was 0.0% and on the period after days 75-76 was only decreased by -2.7%. However, the functional ultimate body weight was reduced less than 10% in both genders, thus considering all these factors, the reduced food consumption is not considered to be adverse.

Table IIA 5.7.4/02–3. Mean overall (Week 1-13) food consumption values, expressed as g/rat/day and (percent change from control)

	Group 1	Group 2	Group 3	Group 4
MALES	**0 ppm**	**100 ppm**	**400 ppm**	**800 ppm**
	21.9	22.1 (+0.9%)	21.4 (- 2.3%)	21.0 (- 4.1%)
FEMALES	**0 ppm**	**100 ppm**	**250 ppm**	**500 ppm**
	17.5	16.7 (- 4.6%)	16.7 (- 4.6%)	15.7 (- 10.3%)

Note: Statistical analysis was not performed on total (week 1-13) food consumption

Data taken from page 35 in the study report

6. Food efficiency: NA

7. Test substance intake: Based on food consumption, body weights, and dietary concentration of test item, the following mean overall (weeks 1 - 13) test item intake was achieved:

Group	Dietary concentration		Nominal test item intake			
	Males	Females	Males		Females	
Nos.	(ppm)	(ppm)	(mg/kg/day)	(ratio)	(mg/kg/day)	(ratio)
1	0	0	---	---	---	---
2	100	100	6.31	1.00	7.48	1.00
3	400	250	25.95	4.11	19.17	2.56
4	800	500	50.67	8.03	37.99	5.08

8. Neurobehavioural Assessment:

a) Functional observational battery (FOB): The only remarkable FOB finding was an impaired pupillary reflex (corresponding to the absent iridic reflex in the left eye noted in the detailed clinical observations) in mid-dose female no. 82 on day 12. This finding was considered incidental to treatment since it occurred with low incidence and frequency, lacked a dose-response relationship, was not observed at subsequent FOB assessment intervals, and represents a typical background finding in rats of this strain and age.

b) Mean body temperature: There were no changes in mean body temperatures that could be conclusively attributed to treatment. No statistically significant changes for body temperature were noted for the females. Statistically significant lower mean body temperatures were noted during weeks 5 and 9 for the 100 and 800 ppm males, but not for the 400 ppm males, relative to controls. No statistically significant changes for body temperature in any of the treated males were noted during weeks 2 and 13. Based on the lower (albeit not statistically significant) mean body temperatures for the 100 and 800 ppm males recorded at acclimatization (prior to initiation of treatment), the transient aspect of these findings, and the lack of a clear dose-response relationship, these alterations in mean body temperature are not considered to be treatment related effects.

c) Grip strength: No treatment-related effects in group mean grip strength were observed during the study. Statistically significant higher mean hind-limb grip strength values during week 5 for the 100 and 800 ppm males, but not the 400 ppm males, relative to controls. Given the relatively low mean male control value at this interval, the transient aspect of this finding, and the lack of a clear dose-response relationship, these increases in mean hind-limb grip strength are not considered to be treatment-related effects.

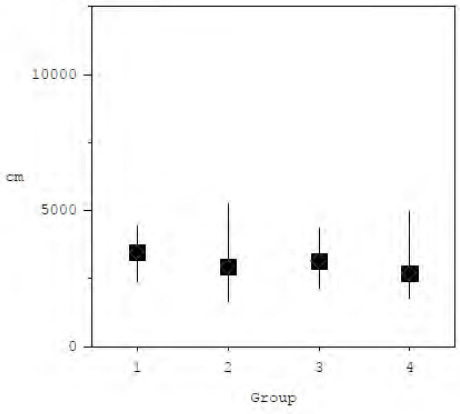
d) Landing foot splay: Landing foot splay was not affected by treatment with the test article.

e) <u>Locomotor activity</u>: No treatment-related effects were observed in locomotor activity testing. Some isolated statistically significant single alterations in distance, center time and/or rearing activity were noted during individual 3-minute intervals (bins) in treated groups, relative to controls. These changes included: 800 ppm males (week 5/distance/bin1, week 5/distance/ bin8, week 13/distance/bin1, week 13/center time/bin2), 400 ppm males (week 5/distance/ bin1), 500 ppm females (week 9/center time/bin5, week 13/center time/bin6), 250 ppm females (week 2/distance/bin1, week2/distance/bin10, week 13/center time/bin6), and 100 ppm females (acclimation/center time/bin5, week 13/center time/bin6, week 9/ rearing/bin1). All these changes occurred at a low incidence and frequency. Since no clear dose response relationship was present and the totals for each parameter showed no statistically significant differences (see below), these findings were considered to represent spurious biological variations rather than findings of any toxicological relevance and not adverse.

AT WEEK 5

MALES

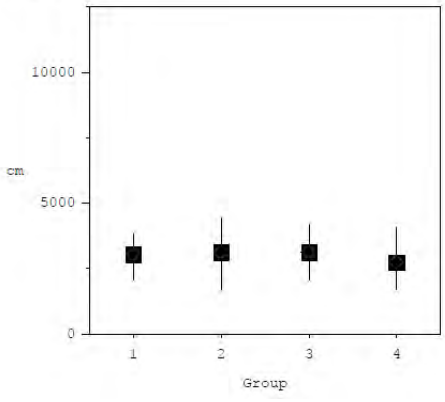
Total distance



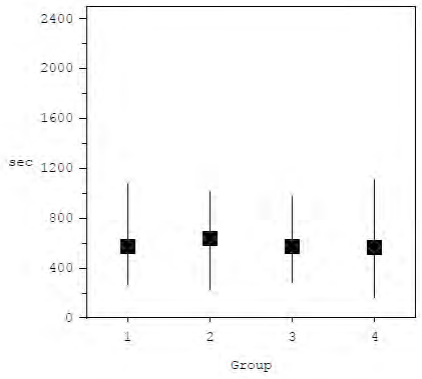
AT WEEK 13

MALES

Total distance



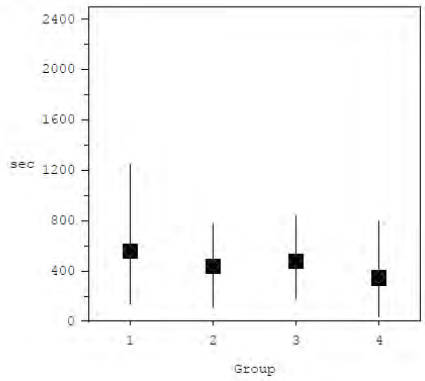
Center time



AT WEEK 9

FEMALES

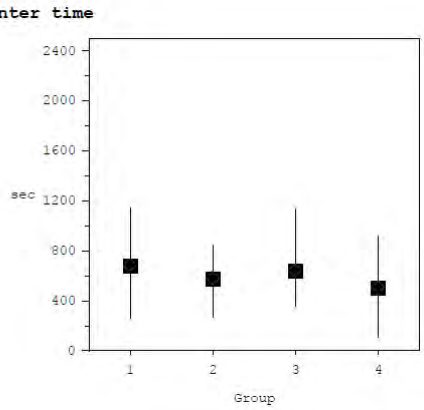
Center time



AT WEEK 13

FEMALES

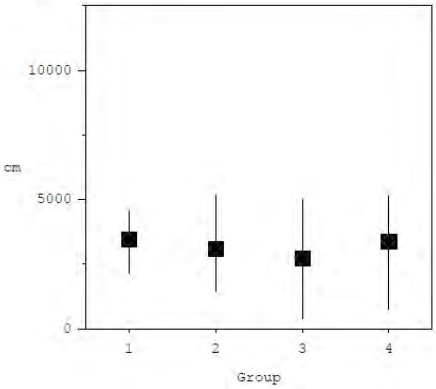
Center time



AT WEEK 2

FEMALES

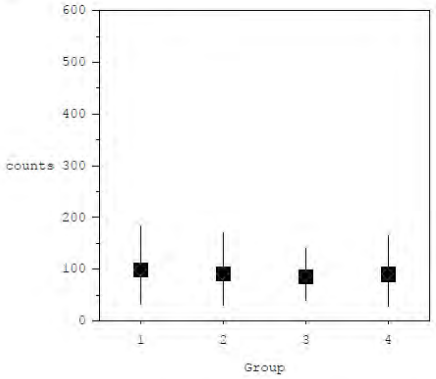
Total distance



AT WEEK 9

FEMALES

Number of rearings



9. Sacrifice and pathology:

a) Gross pathology: There were no gross lesions recorded at necropsy.

b) Brain weight: No treatment-related effects on mean brain weights were evident in males or females. The organ weights adjusted to terminal body weights in the treated male and female groups revealed no statistical differences from controls.

c) Neuropathology: There were no neurohistopathological lesions present that were attributed to treatment with the test article. A peer review of all tissues from selected animals (Male nos. 7, 43 and 44, and Female nos. 45, 91 and 92) was performed which revealed no differences in the diagnosis or gradings recorded by the study pathologist and peer reviewing pathologist. The summary incidence tables and individual animal data sheets listed in the final report are accurately reflecting the mutually agreed-on diagnoses and gradings.

III. Discussion

A. Investigator's conclusions (extracted from page 39 in the study report):

Dietary administration of SYN545192 to Wistar rats for 13 weeks at concentrations of 0, 100, 400, and 800 ppm (males) or 0, 100, 250, and 500 ppm (females) resulted in treatment-related decreases in body weight gain and/or food consumption for the 400 and 800 ppm males and the 500 ppm females. These decreases in body weight gain and food consumption are attributed to generalized system toxicity rather than specific neurotoxic effects. Thus the no-observed-adverse-effect level (NOAEL) for generalized systemic toxicity is 100 ppm for males and 250 ppm for females, corresponding to 6.31 mg/kg body weight/day in males and 19.17 mg/kg body weight/day in females. There was no evidence of neurotoxicity based on the absence of treatment-related clinical observations, ophthalmoscopic findings, FOB findings, locomotor activity changes, and/or neuropathologic findings (i.e, brain weights, macroscopic findings, and microscopic findings). Thus the NOAEL for neurotoxicity was 800 ppm for males and 500 ppm for females, corresponding to 50.67 mg/kg body weight/day in males and 37.99 mg/kg body weight/day in females.

B. Reviewer's conclusions: The reviewer does not concur with the study author's selection of NOAEL and LOAEL values. The ultimate body weight decreases were less than 10% in both genders, thus not considered to be adverse. There were decreases in food consumption and body weight gain, however, they did not have a functional impact on the ultimate body weights in the study, thus not considered adverse. The NOAEL of the study are the highest doses tested, or 50.67 mg/kg/day in males and 37.99 mg/kg/day in females and the LOAEL values were not determined. In the ACN study, the females were more sensitive than males, however in this study, males were more sensitive to the test article than females. The gavage ACN study had effects of decreased body temperature, decreased motor activity and decreased grip strength at 30 mg/kg in females and at 80 mg/kg in males. The dietary administration of the test article may explain the lack of these findings in this sub-chronic study.

C. Deficiencies: Some homogeneity and concentration values exceeded the target range, however, it was not expected to seriously impact the outcome of the study. The control compounds were not tested, nor another study referenced with these results discussed, thus this study is classified as acceptable/non-guideline.

D. References:

1. O. Kempthorne: The design and analysis of experiments. John Wiley, New York (1960)

2. C.W. Dunnett:
A Multiple Comparison Procedure for Comparing Several Treatments with a Control,
J. Amer. Stat. Assoc. 50, 1096-1121 (1955)

3. A.W. Hayes: Principles and Methods of Toxicology (3rd ed.), Raven Press, New York (1984)

4. R.G. Miller: Simultaneous Statistical Interference, Springer Verlag, New York (1981)

STUDY TYPE: Rodent *in vivo* Dermal Absorption Study in rats

TEST MATERIAL: Benzovindiflupyr (BZV)

SYNONYMS: Solatenol

CITATION: S. Runacres and S. Harris, August 15, 2011, SYN545192 EC Formulation (A17056F) – In Vivo Dermal Absorption in the Rat. Final Report. Quotient Bioresearch Limited, Northamptonshire, UK, Report Number SGA/59. Unpublished

SPONSOR: Syngenta Crop Protection, LLC, Greensboro, NC

EXECUTIVE SUMMARY:

Wistar rats were administered nominal doses of 0.001, 0.01 or 1.5 mg/cm^2 of Benzovindiflupyr in an emulsifiable concentrate formulation and monitored up to 120 hr post-dosing. Mean dermal absorption values, for the groups for which the skin site was washed after 6 hours with sacrifice at 120 hrs (n=4), were 17.2%, 7.1% and 12.5% at the three dose levels, respectively.

Recovery of the applied dose (mass balance) was acceptable (group means ranged from 97.78 to 106.27%). Results were not adjusted for incomplete recovery of the applied dose. Values below the limit of quantitation (LOQ) were reported as <0.01. For the purposes of calculations, these values were assumed to be equivalent to half of the LOQ (0.005 % of the applied dose). Values at the limit of detection were assumed to be equivalent to zero for the purposes of the calculations. This may slightly underestimate dermal absorption.

The majority of the administered dose was recovered from the skin wash. Only a small portion of the applied dose was retained at the application site after 120 hours of exposure. Total absorbed dose was calculated by summing residues found in blood, carcass, muscle, liver, kidney, abdominal fat, urine, cage wash, faeces, skin test site, and *stratum corneum*.

The highest proportion of active absorbed was recovered in the faeces by 24 hours. A significant portion was also found in the GI tract and contents, which is consistent with this being the main route of excretion. The amount of active ingredient in the skin at the treatment site (including the *stratum corneum*) decreases with time. This indicates that the proportion of active ingredient in the skin continues to be absorbed over the monitoring period and it was thus included in the estimate of dermal absorption.

Given the uncertainty regarding actual deposition under actual field conditions, it is considered appropriate to derive an estimate of dermal absorption based on the results from the low dose group (0.001 mg/cm^2), as percent dermal absorption was greatest at this dose level. Based on the likely worker exposure time frame, it is considered most appropriate to adopt the dermal absorption value calculated for the group of animals for which the skin site was washed after 6 hours, and which were sacrificed after 120 hrs. Mean dermal absorption for this group of animals was 17.2%. Approximately 1.2% of the applied dose was retained at the skin site.

GUIDELINE OR PROTOCOL FOLLOWED:

1) OECD Guideline 428 (2004). Skin absorption: *In vitro* method
2) EPA (August, 1998) Health Effects Test Guidelines, OPPTS 870-7600 Dermal Penetration
3) European Commission Guidance Document on Dermal Absorption (2004) Sanco/222/2000 rev. 7 (19 March, 2004)
4) OECD Document number 28 (2004b): The conduct of skin absorption studies; Organisation for Economic Cooperation and Development, Paris (ENV/JM/MONO(2004)2.

MATERIALS AND METHODS

MATERIALS

Test Material:

Lot/Batch #: 5173GAR001-1(Radiolabelled); SMU9BP005 (Non-radiolabelled)
Purity: 97.0 % a.i.
CAS #: 1072957-71-1
Structure: $C_{18}H_{15}C_{l2}F_2N_3O$
Vehicle/Solvent used: A17056F Formulations (Emulsifiable Concentrate)
Specific Activity: 5.39 MBq/mg (145.7 µCi/mg)
Radiochemical Purity: 99.7%
Source: Selicia Ltd. (Radiolabelled); Syngenta (Non-radiolabelled)
Other comments:

Relevance of Test Material to Proposed Formulation(s):

The formulation used in this study is an emulsifiable concentrate containing 150 g/L of the active ingredient SYN545192 (Benzovindiflupyr). Details were not presented on the rest of the ingredients in the formulation.

There are several formulations containing benzovindiflupyr proposed for use in Canada. They are formulated as emulsifiable concentrates, soluble liquid and wettable granules. Products contain less than 150 g/L of the active ingredient and 6 of the 8 products are coformulations with difenoconazole, azoxystrobin, or propiconazole.

Test animals:

Species: Rat
Strain: Han Wistar (Wistar CrL: WI(Han))
Gender: Male
Age and weight at study initiation: Age: 6-12 weeks,
Weight: 227-306 g
Source: Charles River UK Ltd, Margate, Kent
Housing: Following application, animals were housed in individual glass metabolism cages.
Diet: Free access; Pellet diet (RM1 (E) SQC, Special Diet Services, Witham Essex, UK) analysed for composition and the presence of contaminants
Water: Free access; domestic water supply, periodically analysed for the presence of contaminants

Environmental conditions
- Temperature: 21 ± 2ºC
- Humidity: 45-65%
- Air changes:
- Photo period: 12 hours light/dark
- Acclimation period: At least 4 days in groups in stock cages after receipt

STUDY DESIGN

Only minor deviations from the study design were reported which are not expected to have a significant impact on the integrity of the study.

Dose

Rationale: Dose selection was based on the formulation concentrate and the 1/150 and 1/1500 aqueous dilutions of the formulation concentrate.

Nominal Doses: 0.001, 0.01, and 1.5 mg ai/cm^2 skin.

Actual Doses: 0.0098, 0.098, and 1.32 mg ai/cm^2 skin (see Table 1 for details)

Actual doses for each animal were calculated by weighing the pipette tip before and after application. The actual dose is a mean of all animals treated for each dose group.

Dose volume: 10 µL/cm^2 skin, 100 µL/animal

Duration of exposures (time from dose to skin wash): 6 hours

Termination periods (time from dose to sacrifice): 6 hours, 24 hours, 72 hours, and 120 hours.

Number of animals/group: 4

Animal Preparation

On the day prior to dosing, the fur behind the shoulders of each rat was clipped and the exposed skin swabbed with acetone to remove sebum. On the day of dosing, each animal was weighed and a dose site definition device (four silicone rubber O-rings glued together) was glued to the clipped skin. Care was taken to avoid the inclusion of any damaged skin within each defined area.

A non-occlusive nylon gauze cover was attached to the top of the dose site definition device after dose application. The total area of skin defined for the dose application to each rat was 10 cm^2 per rat. Following application and attachment of the gauze, the animals were placed in individual glass metabolism cages.

Dose Preparation, Administration and Quantification

Preparation: Dose suspensions were prepared by adding appropriate quantities of [^{14}C]-labelled SYN545192, unlabelled SYN545192 and the blank formulation. The aqueous dilutions were prepared by mixing appropriate quantities of [14C]-labelled SYN545192, unlabelled SYN545192 (1/150 dilution only), blank formulation and water. All dose preparations were formulated at Quotient Bioresearch on the day of first use.

Application: In each experiment the dose preparation was stirred continuously throughout the period of dosing. Following device adhesion, a positive displacement pipette was loaded with the appropriate formulation (10µL/cm^2 and 100 µL per rat), any excess formulation was wiped from the pipette tip with tissue and the pipette was weighed. The 100 µL volume was applied to the defined skin area on each rat and the edge of the pipette tip was used to spread the dispensed dose as evenly as practicable over the defined area, taking care to avoid direct contact of the tip or dose preparation with the application site definition device. The pipette was reweighed so that the weight of the aliquot applied could be calculated. The pipette tips used during the application were separately retained but not analysed, because the pipette weighing procedure was shown to be accurate.

Quantification: The mean radioactivity concentration in each dose preparation was determined by liquid scintillation counting of dilutions of the dose preparation. Samples for [^{14}C]-analysis were taken by weight as dose checks before, during (between each group of four animals) and after the dosing period on each day of use, to ensure homogeneity and to facilitate dose calculations. For each dose check, a positive displacement pipette was loaded with a 100 µL aliquot of formulation, excess formulation was wiped from the pipette tip with tissue and the pipette was re-weighed so that the weight of the aliquot transferred could be calculated. The contents of the volumetric flask were diluted with acetonitrile, with the final weight of the volumetric flask recorded so that the weight of the contents could be calculated. The pipette tips used for the dose checks were separately retained but were not analysed, because the pipette weighing procedure was shown to be accurate. Triplicate weighed aliquots were taken from each dose check flask and separately combined with scintillation fluid prior to radioactivity measurement.

The radiochemical purity of each dose preparation was determined by high performance liquid chromatography (HPLC) before and after each dose session. Data are summarised in Table 6.12.1-1.

Skin Wash (Pre-Sacrifice)

The application site skin of all rats was washed following the 6 hour exposure. The protective gauze cover was removed from the application site definition device and retained. Each application site was washed using nominally 6 pieces of natural sponges pre-wetted with a 3% aqueous soap (Dove liquid) solution and 6 pre-wetted with water, followed by two dry sponges to remove any residual water. Care was taken to avoid the transfer of test substance from the skin surface to the O-ring with the first few sponges and to avoid any loss of the skin washings. All of the sponges used for each rat were retained in a single container for [^{14}C] analysis. The quality of washing was monitored by the use of a Geiger counter to ensure than as much of the residual radiolabelled dose as practicable was removed from the skin surface. If the top two O-rings had been damaged by the animal/removal of the gauze then these were removed and retained with the appropriate gauze. A second nylon gauze cover was used to protect each application site after the 6 hour application site wash and the animals were returned to their appropriate metabolism cages.

Sample Collection

Urine and faeces were collected from each cage at 6 hours and 24 hours after dosing and then at daily intervals for the duration of each experiment. Excreta were stored at approximately -20 ºC prior to analysis. At each sample collection time, each cage was rinsed with a small volume of water and the wash added to the corresponding urine container. At the final sample collection, each cage was washed with ethanol/water 50:50 (v/v) and the washings were separately collected. Cage washings were stored

at ambient temperature. Any urine and faeces excreted during the washing procedure was collected on individual pieces of aluminium foil, and added to the respective 0-6 hour excreta collections.

Each rat was placed on a separate piece of aluminium foil and was anaesthetised using Isoflourane. The second nylon gauze cover (if still attached) was detached and transferred into the appropriate container used for the first nylon gauze cover/upper two rings.

Any residual dose preparation was collected from each application site by washing the skin with liquid soap solution (nominally a 3% aqueous solution of Dove) and water, applied using pieces of pre-wetted natural sponge. Typically 6 sponges of soap solution and 6 with water, followed by two dry sponges to remove any residual water were used to wash each application site, including the inner surface, any remaining O-rings (last few sponges only). Particular care was taken during this procedure to avoid any loss of the skin washings. For each rat, all sponges and washes were collected into a single container. The quality of washing was monitored by the use of a Geiger counter to ensure that as much of the residual radiolabelled dose as practicable was removed from the skin surface. Any remaining dose site definition device (O-rings) were detached and transferred into the appropriate container used for the nylon gauze covers/upper two rings.

Following skin washing, each rat was kept on the appropriate piece of foil and was killed by exsanguination under terminal anaesthesia followed by cervical dislocation. Blood was collected by cardiac puncture and transferred into heparinized tubes. The application site and an annular ring of untreated skin was excised and pinned out on a piece of aluminium foil on a board. The application site area as outlined by the O-rings) was washed and dried with additional soap, water and dry sponges, and the sponges stored with the other terminal sponges for each rat. Using successive pieces of adhesive tape, the application skin site was tape-stripped to remove the *stratum corneum* until the epidermis was visible (the first and second tape strips from each animal were transferred into two separate containers, all subsequent tape strips were paced in a third container). The residual skin and the foil from the board were separately retained for each rat.

The carcass, maintained on the appropriate piece of foil was subjected to tissue sample collection. Any urine present in the bladder was collected and added to the corresponding final urine sample. The gastrointestinal tract and contents, the liver and kidneys, representative samples of muscle and abdominal fat were removed from each rat and separately retained in appropriate pre-labelled containers for analysis. The residual carcass was separately retained and stored along with the tissue samples at approximately -20ºC.

A portion of each whole-blood sample was retained as whole-blood and stored refrigerated, the remaining whole-blood samples were centrifuged for harvesting separate plasma samples which were then stored at approximately -20ºC.

Any urine/faeces collected on the pieces of foil during the washing/sacrifice/tissue collection procedures were transferred to the appropriate final excreta containers. The foil was separately retained for each animal (with the foil from the skin board) but not analysed, because all recoveries of dose were shown to be complete.

Following the removal of the rats and the collection of excreta, metabolism cages were washed with ethanol/water 50:50 (v/v) and the terminal washings were separately collected. Cage washings were stored at ambient temperature.

Sample Preparation and Analysis

All samples were stored frozen for up to 100 days at approximately -20ºC. Details of sample preparation are provided in Table 6.12.1-2. Radioactivity in all samples was quantified directly by liquid scintillation counting, using a Packard liquid scintillation counter with automatic external standard quench correction. After choosing the optimal channel setting, quench correction curves were prepared from radiochemical standards. The validity of the curves was checked throughout the experiments. Radioactivity with less than twice background counts was considered to be below the limit of accurate quantification.

The limit of detection (LOD) of radioactivity in each sample was taken as equal to twice the scintillation counter background rate, in disintegrations per minute, determined by the use of appropriate blanks for each batch of scintillation counts. Since the background varied slightly from batch to batch, the LOD varied accordingly.

Total amounts of radioactivity in samples were reported as a percentage of the total dose and as µg equivalents of SYN545192/g.

RESULTS

Signs and Symptoms of Toxicity

No signs and symptoms of toxicity were presented in the study report.

Summary Tables

Tables 6.12.1-3, 6.12.1-4 and 6.12.1-5 summarize the recovery of applied dose in various media at the high (1.5 mg/cm^2), mid (0.01 mg/cm^2) and low (0.001 mg/cm^2) doses, respectively.

Total Absorbed Dose

Results of analysis are summarised in Tables 6.12.1-3, 6.12.1-4, and 6.12.1-5. Recovery of the applied dose (mass balance) was acceptable (group means ranged from 97.78 to 106.27%). Results were not adjusted for incomplete recovery of the applied dose. Values below the limit of quantitation (LOQ) were reported as <0.01. For the purposes of calculations, these values were assumed to be equivalent to half of the LOQ (0.005 % of the applied dose). Values at the limit of detection were reported as <LOD, but for the sake of calculations were assumed to be equal to zero as LOD values were reported to be variable but were not included in the study report. This may underestimate the dermal absorption value.

The majority of the administered dose was recovered from the skin wash. Only a small portion of the applied dose was retained at the application site after 120 hours of exposure. Total absorbed dose was calculated by summing residues found in blood, carcass, muscle, liver, kidney, abdominal fat, urine, cage wash, faeces, skin test site, and *stratum corneum*.

The highest proportion of active absorbed was recovered in the faeces by 24 hours. A significant portion was also found in the GI tract and contents, which is consistent with this being the main route of excretion. The amount of active ingredient in the skin at the treatment site decreases with time. This indicates that the proportion of active in the skin continues to be absorbed over the monitoring period and it was thus included in the estimate of dermal absorption.

Given the uncertainty regarding actual deposition under actual field conditions, it is considered appropriate to derive an estimate of dermal absorption based on the results from the low dose group (0.001 mg/cm^2), as percent dermal absorption was greatest at this dose level. Based on the likely worker exposure time frame, it is considered most appropriate to adopt the dermal absorption value calculated for the group of animals for which the skin site was washed after 6 hours, and which were

sacrificed after 120 hrs. Mean dermal absorption for this group of animals was 17%. Approximately 1.2% of the applied dose was retained at the skin site.

DISCUSSION

LIMITATIONS OF THE STUDY:

No major limitations were identified.

The exposure duration of 6 hours between application and skin wash may not be representative of the duration of exposure to workers in the field. 10-12 hours would be more representative.

Since the LOD values were variable and not presented in the study report, a value of zero was used to represent values that were reported to be <LOD. This may slightly underestimate dermal absorption, particularly at the low dose.

CONCLUSIONS:

Recovery of the applied dose (mass balance) was acceptable (group means ranged from 97.78 to 106.27%). Results were not adjusted for incomplete recovery of the applied dose. Values below the limit of quantitation (LOQ) were reported as <0.01. For the purposes of calculations, these values were assumed to be equivalent to half of the LOQ (0.005 % of the applied dose). Values at the limit of detection were assumed to be equivalent to zero for the purposes of the calculations. This may slightly underestimate dermal absorption.

The majority of the administered dose was recovered from the skin wash. Only a small portion of the applied dose was retained at the application site after 120 hours of exposure. Total absorbed dose was calculated by summing residues found in blood, carcass, muscle, liver, kidney, abdominal fat, urine, cage wash, faeces, skin test site, and *stratum corneum*.

The highest proportion of active absorbed was recovered in the faeces by 24 hours. A significant portion was also found in the GI tract and contents, which is consistent with this being the main route of excretion. The amount of active ingredient in the skin at the treatment site (including the *stratum corneum*) decreases with time. This indicates that the proportion of active ingredient in the skin continues to be absorbed over the monitoring period and it was thus included in the estimate of dermal absorption.

Given the uncertainty regarding actual deposition under actual field conditions, it is considered appropriate to derive an estimate of dermal absorption based on the results from the low dose group (0.001 mg/cm^2), as percent dermal absorption was greatest at this dose level. Based on the likely worker exposure time frame, it is considered most appropriate to adopt the dermal absorption value calculated for the group of animals for which the skin site was washed after 6 hours, and which were sacrificed after 120 hrs. Mean dermal absorption for this group of animals was 17%. Approximately 1.2% of the applied dose was retained at the skin site.

Table 6.12.1-1 Dosing

Dose Level	Amount compound in dosing solution (mg)		Specific Activity (dpm/µg)	Nominal Dose (mg/cm^2)	Actual Dose (µg/cm^2)
	Radiolabelled	Non-labelled			
concentrate	3.633	597.432	1955	1.5	1.32
1/150	3.614	0.284	299865	0.01	0.0098
1/1500	0.394	0	323454	0.001	0.00098

Table 6.12.1-2 Sample Preparation Details

Sample Media	Preparation Details
Skin Wash	Sponges used to wash application sites were solubilised in Soluene tissue digestant. Samples were mixed with an appropriate scintillant and counted using a liquid scintillation counter.
Faeces	Samples were homogenised to a paste following the addition of a small amount of water. Small sub-samples were analysed by sample oxidation. Weighed aliquots were combusted in oxygen using a Packard automated sample oxidiser. The $^{14}CO_2$ produced was trapped with the carbon dioxide absorbent Carbosorb E$^+$, which was mixed with the scintillant Permafluor E$^+$ prior to liquid scintillation counting. Combustion efficiency was above 95% for all samples oxidised.
Urine	Samples were analysed directly. Samples were mixed with an appropriate scintillant and counted using a liquid scintillation counter.
Whole Blood	Small sub-samples were analysed by sample oxidation. Weighed aliquots were combusted in oxygen using a Packard automated sample oxidiser. The $^{14}CO_2$ produced was trapped with the carbon dioxide absorbent Carbosorb E$^+$, which was mixed with the scintillant Permafluor E$^+$ prior to liquid scintillation counting. Combustion efficiency was above 95% for all samples oxidised.
Cage Wash	Samples were analysed directly. Samples were mixed with an appropriate scintillant and counted using a liquid scintillation counter.
Carcass	Carcass remains were solubilised in tissue digestant (2M sodium hydroxide in distilled water, methanol and Triton X-405) at 55˚C for 48 hours with occasional agitation. Digested samples were mixed with an appropriate scintillant and counted using a liquid scintillation counter.
Nonocclusive Cover (i.e. filter paper)	Dose site definition devices (O-rings and gauze covers) were extracted with acetonitrile. Samples were mixed with an appropriate scintillant and counted using a liquid scintillation counter.

Organs	Gastro-intestinal tract including contents, abdominal fat, kidney, liver and muscle tissue were homogenised by scissor mincing. Small sub-samples were analysed by sample oxidation. Weighed aliquots were combusted in oxygen using a Packard automated sample oxidiser. The $^{14}CO_2$ produced was trapped with the carbon dioxide absorbent Carbosorb E^+, which was mixed with the scintillant Permafluor E^+ prior to liquid scintillation counting. Combustion efficiency was above 95% for all samples oxidised.
Skin at Application Site	*Stratum corneum* on tape strips and application site skin were solubilised in Soluene tissue digestant. Digested samples were mixed with an appropriate scintillant and counted using a liquid scintillation counter.

Table 6.12.1-3 Amount of SYN545192 in each matrix at Specified Hours Post-application of 1.5 mg/cm^2 (skin wash at 6 hours)

Matrix Analysed	Residues in Matrix (% of applied dose)[1]			
	6 h	24 h	72 h	120 h
Non-occlusive cover + enclosure rinse	7.42 (1.77)	7.44 (3.15)	3.97 (3.34)	1.67 (1.44)
Skin Wash (6 hour)	83.00 (2.21)	80.86 (6.63)	80.93 (4.06)	89.57 (9.95)
Skin Wash (terminal)	-	2.91 (1.06)	0.71 (0.91)	0.17 (0.04)
***Stratum Corneum* 1**	0.09 (0.06)	0.07 (0.03)	0.02 (0.03)	0.01 (0.003)
***Stratum Corneum* 2**	0.18 (0.15)	0.06 (0.04)	0.02 (0.03)	0.01 (0.002)
***Stratum Corneum* 3**	0.74 (0.35)	0.29 (0.17)	0.02 (0.01)	0.02 (0.01)
Skin Test Site	9.75 (3.68)	5.20 (1.36)	1.86 (1.07)	0.53 (0.18)
Urine	0.01 (0.00)	0.07 (0.03)	0.74 (0.35)	0.62 (0.21)
Cage Wash	0.24 (0.29)	0.20 (0.19)	1.33 (0.29)	0.66 (0.25)
Faeces	<0.01 (0.01)	0.49 (0.30)	9.69 (5.70)	9.03 (1.90)
Abdominal fat	0.19 (0.23)	0.09 (0.02)	0.10 (0.03)	0.05 (0.03)
Kidney	0.01 (0.003)	0.01 (0.003)	0.02 (0.01)	0.01 (0.01)
Liver	0.06 (0.04)	0.05 (0.03)	0.10 (0.02)	0.05(0.02)
Muscle	0.43 (0.20)	0.25 (0.09)	0.32 (0.10)	0.23 (0.11)
GI tract + contents	0.36 (0.09)	1.22 (0.96)	2.02 (0.48)	0.83 (0.84)
Blood + Carcass	3.81 (1.04)	1.08 (0.53)	1.23 (0.84)	0.47 (0.23)
Recovery (sum of above)	106.27 (3.52)	100.26 (4.41)	103.08 (8.34)	103.92 (9.70)
Dermal Absorption (Based on sum of blood + carcass + muscle + liver + kidney + abdominal fat + urine + cage wash + faeces + skin test site + *stratum corneum*)	15.85 (2.61)	9.05 (2.76)	17.47 (5.79)	12.51 (3.45)

Arithmetic mean values are presented with standard deviations in brackets
[1]mean of 4 animals/group

Table 6.12.1-4 Amount of SYN545192 in each matrix at Specified Hours Post-application of 0.01 mg/cm^2 (skin wash at 6 hours)

Matrix Analysed	Residues in Matrix (% of applied dose)[1]			
	6 h	24 h	72 h	120 h
Non-occlusive cover + enclosure rinse	2.20 (0.88)	2.13 (1.36)	2.59 (1.86)	0.59 (0.29)
Skin Wash (6 hour)	86.92 (4.20)	90.64 (4.03)	83.85 (1.12)	93.17 (2.75)
Skin Wash (terminal)	-	1.71 (0.46)	1.34 (0.48)	0.23 (0.14)
***Stratum Corneum* 1**	0.65 (0.12)	0.08 (0.11)	0.16 (0.06)	0.09 (0.07)
***Stratum Corneum* 2**	0.58 (0.09)	0.07 (0.10)	0.12 (0.08)	0.09 (0.02)
***Stratum Corneum* 3**	0.97 (0.14)	0.17 (0.14)	0.14 (0.11)	0.08 (0.04)
Skin Test Site	1.44 (0.52)	1.29 (0.21)	0.34 (0.23)	0.17 (0.11)
Urine	0.09 (0.04)	0.16 (0.02)	0.80 (0.25)	0.33 (0.03)
Cage Wash	0.02 (0.01)	0.14 (0.11)	0.36 (0.09)	0.26 (0.13)
Faeces	0.01 (0.01)	2.10 (0.56)	9.37 (1.61)	5.82 (0.61)
Abdominal fat	0.36 (0.07)	0.13 (0.04)	0.03 (0.02)	<LOD (N/A)
Kidney	0.05 (0.02)	0.01 (0.01)	0.01 (0.00)	0.0025 (0.029)
Liver	0.29 (0.05)	0.06 (0.01)	0.06 (0.02)	0.01 (0.00)
Muscle	1.31 (0.26)	0.32 (0.19)	0.17 (0.05)	0.01 (0.03)
GI tract + contents	3.83 (1.05)	1.58 (0.07)	0.77 (0.54)	0.11 (0.04)
Blood + Carcass	2.75 (1.64)	0.85 (0.20)	0.50 (0.16)	0.16 (0.04)
Recovery (sum of above)	101.44 (1.64)	101.43 (2.12)	100.60 (0.91)	101.12 (2.48)
Dermal Absorption (Based on sum of blood + carcass + muscle + liver + kidney + abdominal fat + urine + cage wash + faeces + skin test site + *stratum corneum*)	12.32 (3.10)	6.95 (0.98)	12.82 (1.65)	7.13 (0.65)

Arithmetic mean values are presented with standard deviations in brackets
[1]mean of 4 animals/group

Table 6.12.1-5 Amount of SYN545192 in each matrix at Specified Hours Post-application of 0.001 mg/cm^2 (skin wash at 6 hours)

Matrix Analysed	Residues in Matrix (% of applied dose)[1]			
	6 h	24 h	72 h	120 h
Non-occlusive cover + enclosure rinse	4.18 (2.51)	2.46 (0.62)	2.79 (1.63)	1.51 (1.13)
Skin Wash (6 hour)	79.04 (3.68)	75.03 (4.15)	79.19 (2.40)	78.54 (3.64)
Skin Wash (terminal)	-	3.42 (1.09)	1.98 (0.97)	0.53 (0.14)
***Stratum Corneum* 1**	0.37 (0.09)	0.27 (0.25)	0.49 (0.43)	0.29 (0.24)
***Stratum Corneum* 2**	0.22 (0.08)	0.20 (0.19)	0.25 (0.21)	0.15 (0.12)
***Stratum Corneum* 3**	0.46 (0.12)	0.35 (0.29)	0.39 (0.29)	0.29 (0.15)
Skin Test Site	4.59 (0.93)	1.35 (0.83)	0.55 (0.68)	0.45 (0.89)
Urine	0.09 (0.03)	0.50 (0.10)	0.72 (0.30)	0.89 (0.12)
Cage Wash	0.43 (0.86)	0.11 (0.16)	0.30 (0.30)	0.33 (0.24)
Faeces	0.01 (0.01)	8.77 (3.69)	9.77 (1.74)	14.56 (1.67)
Abdominal fat	0.44 (0.17)	0.37 (0.16)	<LOD (N/A)	<LOD (N/A)
Kidney	0.09 (0.04)	0.05 (0.01)	<LOD (N/A)	0.0025 (0.01)
Liver	0.44 (0.12)	0.23 (0.07)	0.05 (0.06)	<LOD (N/A)
Muscle	2.15 (0.45)	1.24 (0.92)	<LOD (N/A)	<LOD (N/A)
GI tract + contents	5.58 (2.16)	5.13 (1.14)	0.97 (0.80)	0.24 (0.20)
Blood + Carcass	2.83 (0.97)	1.51 (0.36)	0.37 (0.45)	0.02 (0.03)
Recovery (sum of above)	100.89 (1.76)	100.97 (0.58)	97.80 (0.14)	97.78 (2.70)
Dermal Absorption (Based on sum of blood + carcass + muscle + liver + kidney + abdominal fat + urine + cage wash + faeces + skin test site + *stratum corneum*)	17.67 (4.41)	20.06 (4.79)	13.85 (2.84)	17.21 (2.05)

Arithmetic mean values are presented with standard deviations in brackets
[1]mean of 4 animals/group

Report:	IIA 5.10/02 Wasil JM (2012). **SYN545192 – A 28-Day Dietary Immunotoxicity Study in CD-1 Female Mice** WIL Research Laboratories, LLC 1407 George Road Ashland, OH 44805-8946 USA, Laboratory Report No. WIL-639155. Issue date: Feb. 16, 2012. Unpublished. MRID #48604461

Guidelines: Immunotoxicity. OPPTS 870.7800 (1998)

Sponsor: Syngenta Crop Protection, LLC 410 Swing Road Post Office Box 18300 Greensboro, NC 27419-8300 USA

Executive Summary: The test substance, SYN545192, was offered *ad libitum* in the diet for 28 consecutive days to female Crl:CD1(ICR) mice at dietary concentrations of 100, 200, and 400 ppm (26.4, 47.1, and 97.1 mg/kg/day), respectively. The concurrent control group (Group 1) and the positive control group (Group 5) were offered the basal diet on a comparable regimen to the SYN545192-treated groups. All mice (Groups 1-5) were immunized with an intravenous injection of sheep red blood cells (sRBC) on study day 24. Mice in the positive control group (Group 5) were administered the positive control substance, cyclophosphamide (CPS), via intraperitoneal injection (50 mg/kg/day) once daily for 4 consecutive days (study days 24 through 27). Each group consisted of 10 mice/group. All animals were euthanized on study day 28. All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed once daily for all animals. Detailed physical examinations were performed approximately weekly, and on the day of the scheduled necropsy. Individual body weights and food consumption values were recorded approximately twice weekly. Complete necropsies were conducted on all animals. The liver, mesenteric lymph node, Peyer's patches, spleen, and thymus were collected at the scheduled necropsy and the liver, spleen, and thymus were weighed. Spleens were placed in Earle's Balanced Salt Solution (EBSS)/HEPES buffer and shipped to ImmunoTox®, Inc. After arrival at ImmunoTox®, Inc., spleen cell suspensions were prepared, spleen cell counts were performed, and the number of specific IgM antibody-forming cells directed towards the sRBC antigen were determined to measure the humoral immune response using the splenic Antibody-Forming Cell (AFC) assay.

All animal survived to the scheduled necropsy. There were no SYN545192-related macroscopic findings or effects on organ weights or food consumption. There were no significant effects on spleen cell number, and SYN545192 did not significantly suppress the humoral immune response when evaluated as either specific activity (AFC/10^6 spleen cells) or as total activity (AFC/spleen) of splenic IgM to the T-cell dependent antigen sRBC. Test substance-related clinical observations of soft feces were often noted in the 400 ppm group throughout the study.

Test substance-related body weight loss was noted in the 400 ppm group on study day 3 compared to the vehicle control group; however, body weight gains were generally similar to the vehicle control group for the remainder of the study. Due to the initial body weight loss from study day 0 to 3, body weights were 8.5% lower on study day 3 compared to the vehicle control group. This initial body weight loss resulted in lower

cumulative body weight gains and/or losses in the 400 ppm group throughout the study. Absolute body weights for this group remained slightly lower throughout the study compared to the vehicle control group, with body weights on study day 28 being 6.6% lower. For the positive control group, CPS, statistically significantly lower spleen weight, spleen cell numbers (-61%), specific activity (-100%), and total spleen activity (-100%) of IgM antibody-forming cells were noted when compared to the vehicle control group. These effects on spleen weights, spleen cell numbers, and spleen activity (*i.e.*, number of specific IgM antibody-forming cells directed towards the sRBC antigen) were consistent with the known immunosuppressant effects of CPS and validated the functionality of the assay.

The NOAEL (no-observed-adverse effect level for the AFC (humoral immune response) assay of 400 ppm (equivalent to 97.1 mg/kg of body weight/day), the highest dose level evaluated. An immunotoxicity LOAEL (lowest observed adverse effect level) was not achieved. The NOAEL for systemic toxicity was 97.1 mg/kg/day and the LOAEL was not achieved.

The immunotoxicity study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a immunotoxicity study (870.7800)

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. No claim of CBI was made for any information contained in this document .

I. Materials and Methods

A. Materials:

Test Material:	SYN545192
Description:	Off white powder
Lot/Batch number:	SMU0FP003 [WIL ID no. 1100B4]
Purity:	97.7%
CAS#:	Not reported
Stability of test compound:	Expiration Date 31 July 2014

Vehicle: PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002 (meal)

Positive Control Material:	Cyclophosphamide monohydrate
Source:	Sigma Aldrich, St. Louis, MO
Lot/Batch number:	079K1569 [WIL ID no. 10019F]
Purity:	>100.0%
Stability:	Expiration date 1 July 2012

Positive Control Substance Vehicle: The vehicle used in preparation of the positive control substance formulation administered to Group 5 was phosphate buffered saline (PBS), prepared using the following components: Phosphate buffered saline, pH 7.4 (lot no. 011M8207, exp. date: 2 March 2012, manufactured by Sigma-Aldrich, Inc., St. Louis, MO) and Sterile water for injection, USP (lot no. C838573, exp. date: 1 June 2012, manufactured by Baxter Healthcare Corporation, Deerfield, IL).

Test Animals:

Species	Female mouse
Strain	Crl:CD1(ICR)
Age at dosing	38 days
Source	Charles, River Laboratories, Inc., Raleigh, NC
Housing	One per cage in stainless steel, wire-mesh cages suspended above cage board
Acclimatisation period	13 days prior to the administration of experimental diets
Diet	PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002 (meal), *ad libitum*
Water	Reverse osmosis-treated (on-site) water *ad libitum*
Environmental conditions	Temperature:22±3°C Humidity: 50±20% Air changes: 10 air changes/hr Photoperiod: 0600-1800 h

B Study Design and Methods:

1. Study experimentation dates – Start: 19 July 2011 End: 27 September 2011

2. Animal assignment and Study Design – Upon arrival, all animals were housed 2 to 3 per cage for approximately 3 days. Thereafter, all animals were housed individually in clean, stainless steel, wire mesh cages suspended above cage board. Animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). On 25 August 2011 (4 days prior to the initiation of basal and test diet administration), all available mice were weighed and examined in detail for physical abnormalities. Based on the review of all appropriate pretest data by the Study Director, which were collected using WTDMS™, animals judged suitable for assignment to the study were selected for use in a computerized randomization procedure based on body weight stratification in a block design. A printout containing the animal numbers and individual group assignments was generated and the animals were then arranged into groups according to the printout. Individual body weights at randomization were within ± 20% of the mean. Each group consisted of 10 females. Individual body weights ranged from 19.2 g to 24.3 g at randomization.

The basal and test diet admix formulations were offered *ad libitum* for 28 consecutive days (beginning on study day 0 and continuing until the time of the scheduled necropsy). The first day of offering the basal or test diets was study day 0. All mice were immunized via an intravenous lateral tail vein injection using an appropriately-sized syringe and needle with 0.2 mL of 1 x 10^8 sRBC (prepared in EBSS with HEPES by WIL Research personnel) on study day 24. The Group 5 mice were administered the positive control substance, CPS, via intraperitoneal injection once daily on study days 24 through 27 at a dose level of 50 mg/kg/day and a dose volume of 10 mL/kg/day. Individual mouse positive control doses were based on the most recently recorded body weights (study day 24) to provide the correct mg/kg/day dose. The sRBC immunizations were administered prior to positive control (CPS) dose administration on study day 24.

Table IIA 5.10/02-1: Study design

Group Number	Treatment [a, b, c]	Dietary SYN545192 Dose Level [a, b] (ppm)	CPS Dose Level [d] (mg/kg/day)	sRBC Dose Volume [c] (mL/mouse)	Number of Female Mice
1	Vehicle Control [a]	0	0	0.2	10
2	Low	100	0	0.2	10
3	Mid	200	0	0.2	10
4	High	400	0	0.2	10
5	Positive Control [a, d]	0	50 [d]	0.2	10

[a] = Mice in Groups 1 and 5 were offered the basal diet (*i.e.*, untreated diet; no SYN545192).

[b] = The SYN545192 dose level is expressed as mg of test substance per kg of diet (ppm) and was not adjusted for body weight or food consumption; the test substance was not adjusted for purity.

[c] = On study day 24, all mice (Groups 1-5) were immunized with an intravenous injection (via lateral tail vein) with 0.2 mL of 1 x 10^8 sheep red blood cells (sRBC) in Earle's Balanced Salt Solution (EBSS) with HEPES. The spleens collected from these immunized mice at the scheduled necropsy were used for the splenic antibody-forming cell (AFC) assay to assess the T-cell dependent antibody response (TDAR).

[d] = Mice in Group 5 were administered cyclophosphamide (CPS), a known immunosuppressant, once daily at 50 mg/kg/day on study days 24 through 27 by intraperitoneal injection. The dose volume was 10 mL/kg/day; the concentration of the dosing formulation was 5 mg/mL. Group 5 served as a positive control and was included to aid in the interpretation of the results and verification of the TDAR assay sensitivity.

3. <u>Dose selection</u> – The dietary concentrations were selected based on the findings of administration of SYN545192 in a 28-day mouse study (Shearer and Wood, 2009) and a 13-week dietary study in the mouse (Mackay and Foster, 2009). Dosages were selected as per agreement with the United States EPA DART for SYN545192 (TXR No. 0055656 dated 10 February 2011). Body weight effects occurred at dietary concentrations of 500 ppm in both studies with some mortality noted in the 13-week study, indicating that this dose was excessive. At 300 ppm, no clear decrease in body weight gain in females occurred by 28 days, but this was observed after 13 weeks as well as gastrointestinal tract hyperplasia. Therefore, the highest dietary concentration selected for the current study was 400 ppm. A dietary concentration of 200 ppm was selected as the mid feeding level for this study. This dietary concentration may produce a decrease in body weight in the first week of the study, and will provide an evaluation of a dose-related response of any effects. No effects were anticipated at the low-diet concentration of 100 ppm, which was determined to be the NOAEL for both the 28-day and 90-day studies in mice.

4. <u>Diet preparation and analysis</u> – PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002 (meal) was used for preparation of the control and test diets. The basal (Groups 1 and 5) and test (Groups 2-4) diets were prepared once or twice weekly, and stored at room temperature. The test diets were not adjusted for purity and were prepared from the lowest to highest concentration. Analyses to determine the homogeneity and stability of the test substance in the diet at 100 and 400 ppm were conducted prior to the dosing phase of the study using test substance diets prepared solely for homogeneity/stability testing. Following preparation of the test diet formulations, samples were collected from the top, middle, and bottom strata of each formulation for homogeneity analysis. Additional samples were collected from the middle stratum and used for stability determinations of the 100 and 400 ppm diets following room

temperature storage under laboratory conditions for 7 and 11 days. Samples were collected from the top, middle, and bottom of each prepared test substance diet admix formulation (including a sample from the middle stratum of the vehicle control diet) during the dosing period. One set of samples from study weeks 0 and 3 were transferred to the WIL Research Analytical Chemistry Department for concentration analysis. Analyses of diet admix formulations during the dosing period were conducted prior to dosing. The remaining set of samples was stored frozen at approximately -20°C as back-up samples to be discarded after acceptance of the analytical results or issuance of the final report. All analyses were conducted by the WIL Research Analytical Chemistry Department using a validated high performance liquid chromatography method using ultraviolet absorbance detection.

Results - Homogeneity Analysis: Prior to dosing, test diet homogeneity was established at 100 and 400 ppm based on the protocol-specified acceptability limits (90% to 110% of the target concentration and had a relative standard deviation [RSD] ≤ 5%) except for the formulation prepared at 400 ppm which had an RSD of 9.2%.

Stability Analysis: The 100 and 400 ppm test diets were stable following up to 11 days of room temperature storage (≥ 90% of initial value).

Concentration Analysis: The analyzed diet admix formulations that were administered to the animals were found to contain 93.5% to 102% of the test substance. The mean concentrations of the test substance in the diet admix formulations used for administration to the animals were within the protocol-specified range (90% to 110% or target concentration and had an RSD ≤ 5%) except for the 100 ppm formulation prepared on 29 August 2011 which had an RSD value of 7.7% and the 100 and 400 ppm formulations prepared on 16 September 2011 which had RSD values of 10% and 5.2%, respectively. The test substance was not detected in the basal diet that was administered to the vehicle and positive control groups (Groups 1 and 5, respectively).

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Positive Control Preparation – The positive control substance, CPS, administered to Group 5 was prepared in a PBS solution at a concentration of 5 mg/mL. The solution was prepared once, divided into at least 4 daily aliquots within a laminar flow hood using sterilized containers and utensils, and stored frozen at approximately -20°C. On each day of dosing (study days 24-27) a vial was quickly thawed, stored on ice, shaken vigorously prior to administration, and administered within 3 hours.

6. Statistics –

All statistical tests were performed using appropriate computing devices or programs. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum

significance levels of 1% and 5%, comparing each SYN545192-treated group to the vehicle control group (Group 1). Each mean was presented with the standard deviation (S.D.), standard error (S.E.), and the number of animals (N) used to calculate the mean. Due to the different rounding conventions inherent in the types of software used, the means and standard deviations on the summary and individual tables may differ by ±1 in the last significant figure. Body weight, body weight change, and food consumption were subjected to a parametric one-way ANOVA (Snedecor and Cochran, 1980) to determine inter-group differences. Following the ANOVA, Dunnett's test (Dunnett, 1964) was used to compare the SYN545192-treated groups (Groups 2-4) to the vehicle control group (Group 1). The positive control group data (Group 5; CPS) were evaluated using the Student′s t-Test (Sokal and Rohlf, 1981) and compared to the vehicle control group (Group 1). Absolute organ weights were analyzed using ANOVA followed by Dunnett′s test (Dunnett, 1964). The adjusted organ weights (*i.e.*, organ weights adjusted based on terminal body weight) were analyzed using ANCOVA followed by Dunnett′s test (Dunnett, 1964; Appendix 8). The SYN545192-treated groups (Groups 2-4) were compared to the vehicle control group (Group 1). The positive control group data (Group 5; CPS) were evaluated using the Student′s t-Test (Sokal and Rohlf, 1981) and compared to the vehicle control group (Group 1). Summary values of organ to terminal body weight ratios for the liver, spleen, and thymus were tabulated but not analyzed statistically.

The terminal body weight, thymus and spleen weights (absolute and relative to terminal body weight), and AFC assay data obtained in this study was analyzed by ImmunoTox®, Inc. In the tables, all data were presented as means with standard deviations. Due to possible differences in rounding procedures, the mean and standard deviation values for the organ weight data generated by ImmunoTox®, Inc., may differ slightly from those generated by WIL Research. The AFC data were expressed as both specific activity, IgM antibody forming cells per million spleen cells (AFC/10_6 spleen cells), and as IgM total spleen activity (AFC/spleen). Data were first tested for homogeneity of variances using the Bartlett′s Chi Square Test (Bartlett, 1937). Homogenous data were evaluated using parametric one-way ANOVA (Kruskal and Wallis, 1952). Following the ANOVA, a Dunnett′s test (Dunnett, 1955; Dunnett, 1964) was performed regardless of whether the ANOVA indicated a statistically significant difference ($p \leq 0.05$). Non-homogenous data were evaluated using a Non-parametric analysis of variance (Wilson, 1956). When significant differences occurred, the treatment groups were compared to the vehicle control group using the Gehan-Wilcoxon Test (Gross and Clark, 1975) as appropriate. The Jonckheere′s Test (Hollander and Wolfe, 1973) was used to test for dose-related trends across the vehicle control and treatment groups. The positive control data were evaluated using the Student′s t-Test (Sokal and Rohlf, 1981) and compared to the vehicle control group. The criteria for accepting the results of the positive control groups was a statistically significant ($p \leq 0.05$) decrease in the response compared to that of the vehicle control group. For the purpose of data interpretation, statistical significance was not

considered automatically to imply immunotoxicological significance. Conversely, the absence of a statistically significant comparison was not considered solely to imply the lack of a biologically relevant effect.

C. Methods –

1. Observations – Clinical examinations were performed once daily for all animals. The absence or presence of findings was recorded for individual animals at the scheduled intervals. Detailed physical examinations were conducted on all animals approximately weekly, beginning at least 1 week prior to randomization, at the time of randomization, and on the day of the scheduled necropsy. Clinical observations were not performed on days when detailed physical examinations were conducted. A separate computer protocol was used to record any treatments required during the study.The investigators did not specify as to whether the modified FOB for immunotox studies was followed.

2. Body weight – Individual body weights were recorded approximately twice weekly throughout the study, beginning approximately 1 week prior to randomization, at the time of randomization, on study day 0, and ending just prior to the scheduled necropsy. Mean body weights and mean body weight changes were calculated for the corresponding intervals.

3. Food consumption and test substance intake – Individual food consumption was recorded approximately twice weekly, beginning approximately 1 week prior to randomization, and ending just prior to the scheduled necropsy. Food intake was calculated as g/animal/day for each interval. When food consumption could not be measured for a given interval (due to spillage, weighing error, obvious erroneous value, *etc.*), the appropriate interval was footnoted as "NA" on the individual tables. The mean amounts of SYN545192 consumed (mg/kg of body weight/day) by each treatment group were calculated from the mean food consumed (g of food/kg of body weight/day using the average of the first and last body weight during the week) and the appropriate target concentration of SYN545192 in the food (mg of test substance/kg of food).

4. Clinical Chemistry – Blood samples were collected at the time of euthanasia via the inferior vena cava of animals euthanized by inhalation of carbon dioxide. The blood samples were collected into tubes containing potassium EDTA as an anticoagulant for the preparation of blood smears for possible future evaluation.

5. Sacrifice and Pathology – Animals were sacrificed by carbon dioxide inhalation on day 28.

a. Gross necropsy – A complete necropsy was conducted on all animals at the scheduled necropsy. The necropsies included, but were not limited to, examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities, including viscera.

b. Organ weights – From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

x	liver	x	thymus
x	spleen[a]		

a = Spleen "wet" weights were recorded at the scheduled necropsy (see Section 3.4.5).

c. Tissue preparation/histology – The following tissues were examined *in situ*, removed and examined and fixed in 10% neutral buffered formalin (except as noted):

X	bone marrow smear[a] right femur	X	peyer's patches (GALT)
X	liver (appropriate sections for possible histopathologic assessment)	X	spleen[b]
X	lymph node mesenteric	X	thymus

[a] Bone marrow smears were obtained at necropsy; slides were not examined.
[b] Placed in EBSS/HEPES buffer solution

Tissues were stored as appropriate for future histopathological examination.

6. Immunotoxicity –

a. Functional Activity –

IgM antibody analysis –
Blood samples were collected at the time of euthanasia via the inferior vena cava of animals euthanized by inhalation of carbon dioxide. The blood samples were collected into borosilicate glass tubes and processed to obtain serum samples. The serum was transferred to cryovials and stored frozen at approximately -70ºC at WIL Research for possible future IgM antibody analysis.

Spleen processing for immunotoxicological evaluation –
Spleens were collected from all animals at the scheduled necropsy immediately following blood collection. Individual spleens were placed into individual preweighed (tared) tubes (maintained on ice) containing EBSS with 15 mM HEPES and supplemented with gentamicin as a bacteriostat. Each tube was then weighed to provide a "wet" weight for each spleen. Spleen samples from Group 1-4 animals were randomized and coded for AFC analysis. This was done to ensure that the analyst was unaware from which treatment group the spleen sample had been collected. Spleen samples from Group 5 were labelled as positive control samples for analysis. The spleen samples were placed on crushed ice and shipped to ImmunoTox®, Inc., via overnight courier for AFC analysis. Upon receipt at ImmunoTox®, Inc., the spleens were accessioned in accordance with the ImmunoTox®, Inc., SOP for receipt of biological samples. Spleen, thymus, and terminal body weight data previously recorded by WIL Research was provided to ImmunoTox®, Inc., for subsequent organ weight analysis. The spleen samples were processed into single-cell suspensions prepared in accordance with the ImmunoTox®, Inc., SOP for mouse spleens. The cell suspensions were centrifuged and resuspended in EBSS with HEPES. Spleen cell counts were performed using a Model Z1 Coulter Counter®. Viability of splenocytes was determined using propidium iodide and the Coulter® EPICS® XL-MCL Flow Cytometer. The AFC assay served to determine the number of specific IgM antibody forming cells directed towards sRBC and was a

modification of the Jerne plaque assay (Jerne and Nordin, 1963; Jerne *et al.*, 1974; White *et al.*, 2010).

II. RESULTS

A. Observations –

1. Clinical signs of toxicity – Clinical observations of soft feces were often noted in the 400 ppm group throughout the study, beginning as early as study day 5. All other clinical findings in the SYN545192-treated groups were noted with similar incidence in the vehicle control group, were limited to single animals, were not noted in a dose-related manner, and/or were common findings for laboratory mice of this age and strain.

2. Mortality – All animals survived to the scheduled necropsy.

B. Body weight and weight gain – SYN545192-related mean body weight loss was noted in the 400 ppm group on study day 3 compared to the vehicle control group; however, mean body weight gains were generally similar to the vehicle control group for the remainder of the study. Due to the initial body weight loss from study day 0 to 3, mean body weights were 8.5% lower on study day 3 compared to the vehicle control group. This initial body weight loss resulted in statistically significantly lower mean cumulative body weight gains and/or losses in the 400 ppm group from study days 0 to 3, 0 to 7, 0 to 14, and 0 to 28. Absolute mean body weights for this group remained slightly lower throughout the study compared to the vehicle control group, with mean body weights on study day 28 being 6.6% lower. Since the body weight changes were less than 10%, the test article effects on body weight and body weight gain are not considered adverse. Statistically significantly higher mean body weight gains were noted in the 200 ppm group from study day 3 to 7; however, this difference was considered incidental and not related to SYN545192 administration due to the lack of a similar trend at other intervals. The positive control group (Group 5) had a statistically significantly lower mean body weight on study day 28 and a statistically significantly lower mean cumulative body weight gain from study day 0 to 28 compared to the vehicle control group.

Table IIA 5.10/02-3. Average body weights during 28 days of treatment

	FEMALES				
GROUP:	0 PPM	100 PPM	200 PPM	400 PPM	50 MG/KG CPS
DAY 3					
MEAN	23.4	22.2	23.1	21.4b	22.9
% DIFFERENCE		-5.1	-1.3	-8.5	-2.1
S.D.	1.18	1.59	1.33	1.57	1.75
S.E.	0.37	0.50	0.42	0.50	0.55
N	10	10	10	10	10
7					
MEAN	23.0	22.2	23.5	21.5	22.9
% DIFFERENCE		-3.5	2.2	-6.5	-0.4
S.D.	1.44	1.54	1.39	1.84	1.90
S.E.	0.46	0.49	0.44	0.58	0.60
N	10	10	10	10	10
10					
MEAN	23.5	22.6	24.2	22.5	23.4
% DIFFERENCE		-3.8	3.0	-4.3	-0.4
S.D.	1.54	1.42	1.38	1.27	1.63
S.E.	0.49	0.45	0.44	0.40	0.51
N	10	10	10	10	10
14					
MEAN	24.0	22.8	24.5	22.8	23.3
% DIFFERENCE		-5.0	2.1	-5.0	-2.9
S.D.	1.57	1.46	1.49	1.42	1.77
S.E.	0.50	0.46	0.47	0.45	0.56
N	10	10	10	10	10

STATISTICAL ANALYSES PERFORMED USING AN ANOVA AND DUNNETT'S TEST (GROUPS 2, 3, 4) OR STUDENT'S T-TEST (GROUP 5)
For statistical analyses, control group 1 was compared to groups 2, 3 and 4; control group 1 was compared to group 5.
b = Significantly different from control group 1 at 0.01 using Dunnett's test

	FEMALES				
GROUP:	0 PPM	100 PPM	200 PPM	400 PPM	50 MG/KG CPS
DAY 17					
MEAN	24.9	24.1	25.4	23.8	24.5
% DIFFERENCE		-3.2	2.0	-4.4	-1.6
S.D.	1.80	1.62	1.59	1.98	1.91
S.E.	0.57	0.51	0.50	0.62	0.60
N	10	10	10	10	10
21					
MEAN	25.5	24.7	26.1	24.3	24.9
% DIFFERENCE		-3.1	2.4	-4.7	-2.4
S.D.	1.83	1.74	1.70	2.06	1.94
S.E.	0.58	0.55	0.54	0.65	0.61
N	10	10	10	10	10
24					
MEAN	26.6	25.6	26.7	25.3	25.6
% DIFFERENCE		-3.8	0.4	-4.9	-3.8
S.D.	1.67	1.99	2.08	2.24	1.89
S.E.	0.53	0.63	0.66	0.71	0.60
N	10	10	10	10	10
28					
MEAN	27.4	26.0	27.5	25.6	25.4c
% DIFFERENCE		-5.1	0.4	-6.6	-7.3
S.D.	1.87	2.04	2.17	2.17	1.77
S.E.	0.59	0.64	0.69	0.69	0.56
N	10	10	10	10	10

STATISTICAL ANALYSES PERFORMED USING AN ANOVA AND DUNNETT'S TEST (GROUPS 2, 3, 4) OR STUDENT'S T-TEST (GROUP 5)
For statistical analyses, control group 1 was compared to groups 2, 3 and 4; control group 1 was compared to group 5.
c = Significantly different from control group 1 at 0.05 using Student's t-Test

[a] Data obtained from pages 41-42 in the study report.

C. Food consumption and compound intake –

1. Food consumption - Food consumption was unaffected by test article administration.

2. Compound consumption – The average achieved SYN545192 consumptions (mg/kg of body weight/day) over 28 days of treatment based on target dietary concentrations of SYN545192, body weight, and food consumption data, are presented below.

Average SYN545192 Consumptions (mg/kg/day)

Targeted Dietary Level (ppm)	Average SYN545192 Consumption (mg/kg of body weight/day)
100	26.4
200	47.1
400	97.1

D. Gross Necropsy –

1. Organ weight – There were no statistically significant differences in absolute or relative liver, spleen, or thymus weights when the test substance-treated groups were compared to the vehicle control group. The spleen weight relative to body weight increased by 12.7%, but the effect was not dose responsive, nor statistically significant. The thymus weight relative to body weight decreased by 14.7%, but the effect was not dose responsive, nor statistically significant. The positive control group had statistically significantly lower spleen and thymus weights compared to the vehicle control group.

Table IIA 5.10/02-4. Select organ weights[a]

	FEMALES				
GROUP:	0 PPM	100 PPM	200 PPM	400 PPM	50 MG/KG CPS
FINAL BODY WT (G)					
MEAN	27.4	26.0	27.5	25.6	25.4c
% DIFFERENCE		-5.1	0.4	-6.6	-7.3
S.D.	1.87	2.04	2.17	2.17	1.77
S.E.	0.59	0.64	0.69	0.69	0.56
N	10	10	10	10	10
LIVER (G)					
MEAN	1.4152	1.3110	1.3968	1.3014	1.3667
% DIFFERENCE		-7.4	-1.3	-8.0	-3.4
S.D.	0.15820	0.15450	0.19491	0.14961	0.18348
S.E.	0.05003	0.04886	0.06164	0.04731	0.05802
N	10	10	10	10	10
LIVER (G/100 G FINAL BODY WEIGHT)					
MEAN	5.165	5.045	5.069	5.090	5.371
% DIFFERENCE		-2.3	-1.9	-1.5	4.0
S.D.	0.4485	0.3642	0.4782	0.4544	0.4082
S.E.	0.1418	0.1152	0.1512	0.1437	0.1291
N	10	10	10	10	10
ADJUSTED LIVER WEIGHT (G)					
MEAN	1.3703	1.3488	1.3457	1.3596	NA
MEAN	1.3409	NA	NA	NA	1.4410

STATISTICAL ANALYSES PERFORMED USING AN ANOVA AND DUNNETT'S TEST (GROUPS 2, 3, 4) OR STUDENT'S T-TEST (GROUP 5)
For statistical analyses, control group 1 was compared to groups 2, 3 and 4; control group 1 was compared to group 5.
c = Significantly different from control group 1 at 0.05 using Student's t-Test
NA = NOT APPLICABLE

GROUP:	0 PPM	100 PPM	200 PPM	400 PPM	50 MG/KG CPS
		FEMALES			
SPLEEN- WET (G)					
MEAN	0.1312	0.1184	0.1225	0.1385	0.0737d
% DIFFERENCE		-9.8	-6.6	5.6	-43.8
S.D.	0.01279	0.02763	0.03815	0.04782	0.02151
S.E.	0.00404	0.00874	0.01206	0.01512	0.00680
N	10	10	10	10	10
SPLEEN- WET (G/100 G FINAL BODY WEIGHT)					
MEAN	0.479	0.454	0.445	0.540	0.288
% DIFFERENCE		-5.2	-7.1	12.7	-39.9
S.D.	0.0331	0.0830	0.1271	0.1780	0.0762
S.E.	0.0105	0.0262	0.0402	0.0563	0.0241
N	10	10	10	10	10
ADJUSTED SPLEEN WEIGHT (G)					
MEAN	0.1258	0.1229	0.1164	0.1455	NA
MEAN	0.1249	NA	NA	NA	0.07988-D

STATISTICAL ANALYSES PERFORMED USING AN ANOVA AND DUNNETT'S TEST (GROUPS 2, 3, 4) OR STUDENT'S T-TEST (GROUP 5)
For statistical analyses, control group 1 was compared to groups 2, 3 and 4; control group 1 was compared to group 5.
d = Significantly different from control group 1 at 0.01 using Student's t-Test
D = Adjusted organ weight significantly different from control group 1 at 0.01 using Student's t-Test
NA = NOT APPLICABLE

GROUP:	0 PPM	100 PPM	200 PPM	400 PPM	50 MG/KG CPS
		FEMALES			
THYMUS (G)					
MEAN	0.0518	0.0490	0.0439	0.0413	0.0175d
% DIFFERENCE		-5.4	-15.3	-20.3	-66.2
S.D.	0.00857	0.01359	0.01722	0.00957	0.00282
S.E.	0.00271	0.00430	0.00545	0.00303	0.00089
N	10	10	10	10	10
THYMUS (G/100 G FINAL BODY WEIGHT)					
MEAN	0.190	0.188	0.157	0.162	0.069
% DIFFERENCE		-1.1	-17.4	-14.7	-63.7
S.D.	0.0328	0.0483	0.0495	0.0357	0.0121
S.E.	0.0104	0.0153	0.0157	0.0113	0.0038
N	10	10	10	10	10
ADJUSTED THYMUS WEIGHT (G)					
MEAN	0.04936	0.05102	0.04115	0.04442	NA
MEAN	0.05162	NA	NA	NA	0.01760-D

STATISTICAL ANALYSES PERFORMED USING AN ANOVA AND DUNNETT'S TEST (GROUPS 2, 3, 4) OR STUDENT'S T-TEST (GROUP 5)
For statistical analyses, control group 1 was compared to groups 2, 3 and 4; control group 1 was compared to group 5.
d = Significantly different from control group 1 at 0.01 using Student's t-Test
D = Adjusted organ weight significantly different from control group 1 at 0.01 using Student's t-Test
NA = NOT APPLICABLE

[a] Data obtained from page 53-55 in the study report.

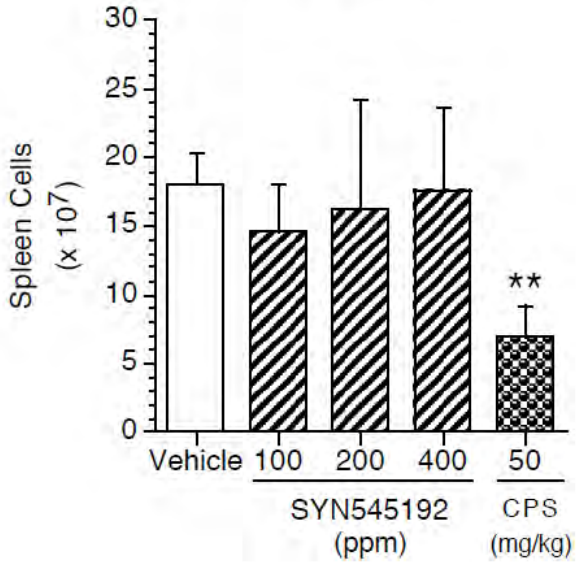
2. <u>Gross necropsy and histology</u> – There were no test article-related macroscopic findings.

E. <u>Immunotoxicity Tests</u> –

1. Functional Activity –

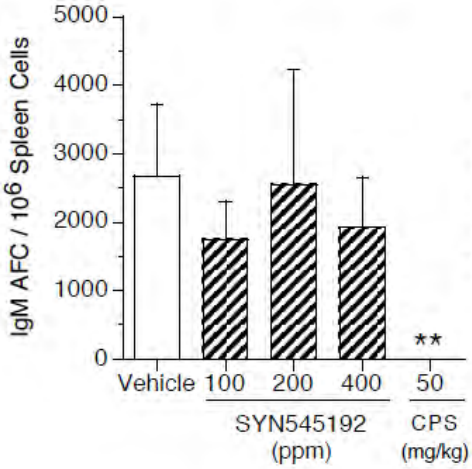
a. Antibody forming cell (AFC) assay – There were no statistically significant effects on spleen cell number at any dose level of the test article. In the functional evaluation of the IgM AFC response, treatment with the test article did not result in statistically significant effects on the humoral immune response when evaluated as either specific activity (AFC/106 spleen cells) or as total spleen activity (AFC/spleen), compared to the vehicle control group (mean +/- SD result graphs below[a]). A statistically significant lower spleen cell number (61%), specific activity (-100%), and total spleen activity (-100%) were noted in the positive control (CPS) group when compared to the vehicle control group.

Spleen Cell Number in Female CD-1 Mice Exposed to SYN545192 via Diet for 28 Days

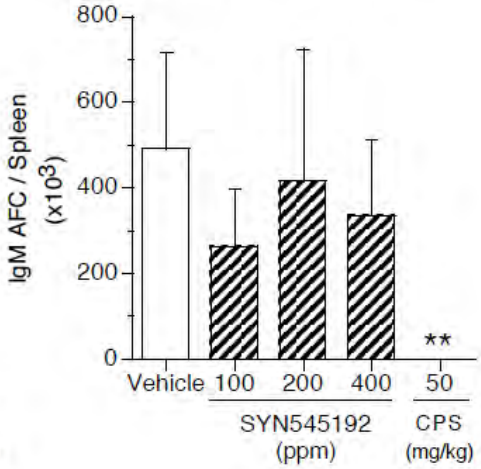


IgM Antibody-Forming Cell Responses to Sheep Erythrocytes in Female CD-1 Mice Exposed to SYN545192 via Diet for 28 Days – Day 4 Response

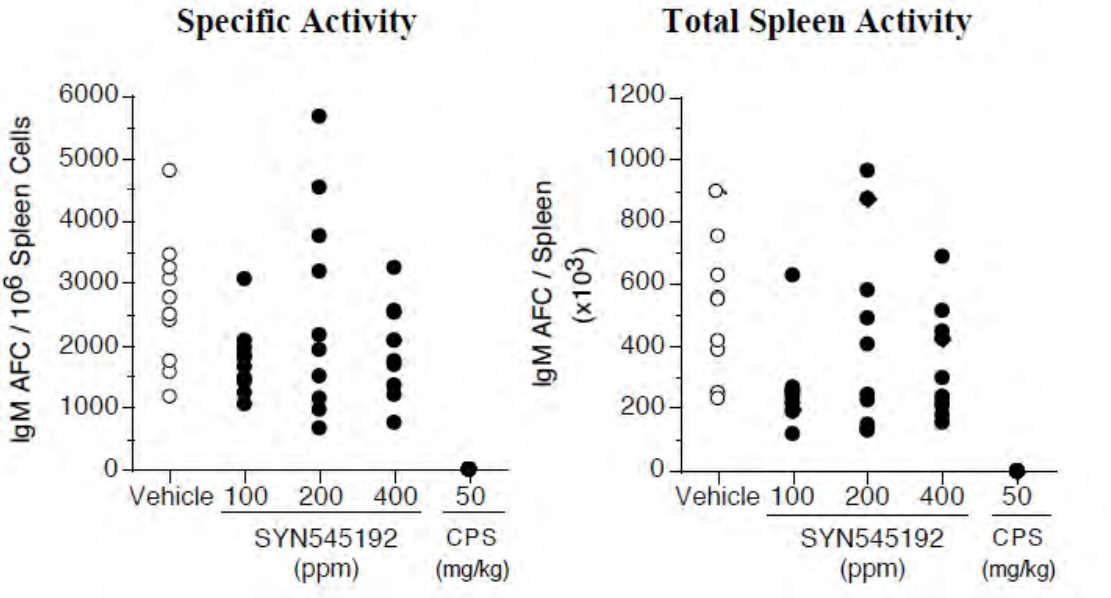
Specific Activity



Total Spleen Activity



Individual IgM Antibody-Forming Cell Responses to Sheep Erythrocytes in Female CD-1 Mice Exposed to SYN545192 via Diet for 28 Days – Day 4 Response



[a] Data obtained from page 12-129 of the study report
* Statistically significant difference from control group mean, $p<0.05$ (Dunnett's test (H) or non-parametric tests (NH))
** Statistically significant difference from control group mean, $p<0.01$ (Dunnett's test (H) or non-parametric tests (NH)))

III. Discussion

A. <u>Investigator's Conclusions (extracted from page 31 in the study report)</u> –

Based upon the results of this study, SYN545192 administered *ad libitum* in the diet for 28 consecutive days to female Crl:CD1 (ICR) mice at dose levels of 100, 200, and 400 ppm resulted in a no-observed-adverse effect level (NOAEL) for the AFC (humoral immune response) assay of 400 ppm (equivalent to 97.1 mg/kg of body weight/day), the highest dose level evaluated. The LOAEL value was not achieved.

B. <u>Reviewer's Conclusions</u> – The Agency concurs with the study author's conclusion that the immunotoxicity NOAEL is 400 ppm or 97.1 mg/kg/day, based on the AFC results presented did not achieve statistical significance. The cyclophosphamide control showed positive results to suppress the immune response, however the 50 mg/kg/day dose seemed to be excessive to demonstrate immune supression (i.e. a 100% decrease in the AFC response). The AFC specific activity decreased approximately 25% at 400 ppm SYN545192, however, the effect was not statistically significant with the coefficient of variation in the assay.

C. <u>Deficiencies</u> – The immunotoxicity AFC results with p-values should have been shown in tables in addition to the graphs. The coefficient of variation of the AFC specific activity was as high as approximately 65% in some test groups (i.e. 200 ppm SYN545192). However, here were no study deficiencies that would apparently affect the outcome of the study.

Report:	IIA 5.5.4/04. Robertson B, 2012b. SYN545192: 14 day dietary thyroid mode of action study in rats with a 63 day recovery period. Charles River Laboratories, Preclinical Services, Tranent (PCS-EDI), Edinburgh, EH33 2NE, UK. Laboratory Report No. 33367. 13 July 2012. Unpublished (Syngenta File No.SYN545192_10222). EPA MRID No. 48604553

Guidelines: There is no applicable test guideline for an investigative toxicity study of this type.

GLP: Signed and dated GLP and Quality Assurance statements were provided. No claim of compliance was made for the Cell Proliferation work performed at CXR Biosciences Ltd., Dundee Technopole, Dundee, UK.

EXECUTIVE SUMMARY

The purpose of this study was to evaluate the effects of SYN545192 treatment on a number of liver and thyroid parameters in order to investigate the mode of action for the increased incidence of thyroid follicular cell adenomas observed in male Han Wistar rats at the top dose level (600 ppm) in a 2 year carcinogenicity study (***Charles River Study No. 459580***).

Groups of 60 male Han Wistar Crl: WI(Han) rats were assigned to the main study and fed diets containing 100, 600 or 1200 ppm of SYN545192 for a period of up to 14 consecutive days. The control group received blank diet for the same duration as the treated animals. An additional group of 60 male rats were fed diets containing phenobarbital sodium salt at 1200 ppm to act as a positive control for the liver and thyroid parameters investigated.

A further 2 groups of 15 males were assigned to a recovery study and dosed at 0 or 1200 ppm SYN545192 for 14 consecutive days followed by a 63 day recovery period.

The following were assessed at predetermined intervals from pre-trial until study completion for all animals: viability, clinical observations, body weights, food and water consumption. Blood samples were also collected from all animals at each scheduled euthanasia time-point for thyroid function testing.

All main study animals were terminated after completion of 1, 3, 7, or 14 days of treatment (i.e. days 2, 4, 8 or 15) and all recovery study animals were terminated after a subsequent 63 day recovery period (day 78). Each animal underwent a detailed necropsy examination, and the liver and thyroid glands were weighed. The liver, thyroid glands and gross abnormalities from all animals were subjected to histological evaluation. 5-Bromo-2'- deoxyuridine (BrdU) was administered to each animal two hours before termination, and cell proliferation in the thyroid by BrdU labelling index was measured. Frozen samples of the liver from each animal were analysed for hepatic microsomal protein content and UDPglucuronosyltransferase (UDPGT) activity with thyroxine (T_4) as substrate.

Treatment with SYN545192 resulted in dose- and/or time-related effects on a number of parameters associated with the liver and/or thyroid.

Treatment with 100 ppm SYN545192 resulted in increased hepatic UDPGT activity (using thyroxine as a substrate) on day 4 and decreased serum T_3 and increased hepatic microsomal

protein content on day 15 compared to control. As these changes occurred at isolated time points only, the relevance to the mode of action is unknown.

Treatment with 600 ppm SYN545192 resulted in decreased body weight gains, decreased food consumption, increased liver weight (day 15), increased incidence of centrilobular hepatocyte hypertrophy, decreased serum T_3, increased TSH, increased hepatic UDPGT activity and increased hepatic microsomal protein content.

Treatment with 1200 ppm SYN545192 resulted in increased liver weight, increased incidence of centrilobular hepatocyte hypertrophy, increased hepatic UDPGT activity, increased hepatic microsomal protein content, decreased serum T_3, decreased serum T_4, increased serum TSH, increased thyroid weight and increased thyroid follicular cell proliferation by BrdU incorporation. All of these effects were fully reversed after a 63 day recovery period following 14 days of treatment.

Treatment with 1200 ppm phenobarbital sodium salt resulted in increased liver weight, increased incidence of centrilobular hepatocyte hypertrophy, increased hepatic UDPGT activity towards thyroxine as a substrate, increased hepatic microsomal protein content ,decreased serum T_3, decreased serum T_4, increased serum TSH, increased thyroid weight, increased thyroid follicular cell hypertrophy and increased thyroid follicular cell proliferation by BrdU incorporation.

This study has demonstrated that dietary treatment of male rats with SYN545192 results in increased hepatic UDPGT activity, hepatocellular centrilobular hypertrophy, increased liver weight, decreased serum T_3 and T_4, and increased serum TSH associated with increased thyroid follicular cell replicative DNA synthesis and thyroid weight. These effects can be used as part of a weight-of-the-evidence approach to explain the mode of action for the increased incidence of thyroid follicular cell adenomas observed in male Han Wistar rats in a 2 year carcinogenicity study (*Charles River Study No. 459580*).

MATERIALS AND METHODS

Materials:

Test Material:	SYN545192
Description:	Technical, beige powder
Lot/Batch number:	SMU9BP005
Purity:	97.0% a.i
CAS#:	1072957-71-1
Stability of test compound:	Recertification date: End of February 2013

Vehicle and/or positive control: The test substance was administered via Rat and Mouse (modified) No.1 Diet SQC Expanded Ground. The positive control substance was Phenobarbital Sodium Salt (100% purity; Lot number 031M1417V; Sigma-Aldrich UK)

DNA Labelling Reagent: 5-Bromo-2'-deoxyurudine (BrdU)
Description: White powder
Lot/Batch number: HMBB9193V
Purity: 100%
CAS#: Not given
Stability of test compound: No expiry date given

Test Animals:

Species	Rat
Strain	Crl:WI(Han)
Age/weight at dosing	Approximately 6-7 weeks/152-239 g males
Source	Charles River UK Ltd., Margate, Kent, UK
Housing	Animals were housed 3 per cage by sex in polycarbonate cages (61 x 43.5 x 24 cm) with stainless steel grid tops, solid bottoms and an integral food hopper.
Acclimatisation period	3 weeks
Diet	Rat and Mouse (modified) No.1 Diet SQC Expanded Ground *ad libitum*
Water	Public water supply *ad libitum*
Environmental conditions	Temperature: 20-23°C Humidity: 54-60% Air changes: Approximately 15 changes/hour Photoperiod: 12 hrs. light/12 hours dark

Study Design and Methods:

In-life dates: Start: 06 January 2012 End: 21 May 2012

Animal assignment: Cages were racked by treatment group and vertically throughout the rack. Control animals were housed on a separate rack. During pre-trial, group mean body weights were checked to ensure all groups were within ±20% of the mean body weight of all animals in the study. Group means were found to be within 1% of the mean weight of all animals in the study.

Table B.6.8.1/04-1: Study design

Group	Test substance	Treatment (ppm)	Treatment (mg/kg bw per day)	Main study (numbers)				Recovery (numbers)
				Timepoint (day)				
				2	4	8	15	
1	Vehicle	0	0	15	15	15	15	15
2	SYN545192	100	9.9	15	15	15	15	-
3	SYN545192	600	57.7	15	15	15	15	-
4	SYN545192	1200	112.8	15	15	15	15	15
5	Phenobarbital Sodium Salt (PB)	1200		15	15	15	15	

Diet preparation and analysis: Diet formulations were prepared from a 5000 ppm stock premix. This was prepared by making a 200 g premix which contained the total weight of test

substance required to produce the final concentration and amount of diet requested and untreated control diet. This was mixed in an automated mortar and pestle and ground for 5 minutes. Before the test substance was weighed, it was ground in a mortar and pestle and sieved through a 0.5 mm mesh sieve. The premix was then blended with the required amount of untreated diet and mixed for 20 minutes in a diet mixer. The test diets were prepared from a dilution of the stock concentration group by adding an appropriate amount of 5000 ppm diet to the appropriate weight of blank RM1 control diet. Diets were mixed for 20 minutes in a diet mixer. Formulated diets were prepared weekly.

Diet formulations (without the test substance SYN545192) were prepared for the control animals.

Diet formulations for the positive control substance (1200 ppm PB) were prepared weekly.

Samples from diets were collected for analysis for concentration and homogeneity on day 1 and week 2.

Concentration analysis results: The concentrations at all time points analysed were within acceptable limits (±10% of nominal) indicating acceptable accuracy of formulations.

Homogeneity results: The coefficient of variation was low (<10%) indicating acceptable homogeneity of formulations.

SYN545192 was not detected in the control diet samples.

Observations: All animals were checked early morning and as late as possible each day for viability. Additionally, all animals were examined for reaction to treatment twice each day from day 2 onwards. The onset, intensity and duration of any signs were recorded.

Once each week, beginning during the week of the pre-trial period, each animal was removed from the cage and received a detailed clinical examination including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.

Body weight: Body weights were recorded once during the pre-trial period, and twice weekly during the dosing and recovery period, including day of terminal kill.

Food consumption and test substance intake: The quantity of food consumed by each cage of animals was measured and recorded once during the pre-trial period, then twice weekly during the dosing and recovery periods including day of terminal kill.

The amount of test substance ingested was calculated for each cage (groups 2-4) of animals scheduled for termination on treatment day 15 and recovery day 63 over every period of food consumption during treatment.

Water consumption: Water consumption was qualitatively monitored by visual inspection of the water bottles on a weekly basis during the study

Clinical pathology

Sample collection for thyroid function testing: Blood samples (approximately 2.0 mL) were collected from the orbital sinus under isofluorane anaesthesia. The animals were not fasted prior to collection. Only the animals designated for euthanasia were sampled on the scheduled days as detailed below:

Sample collection time points

Group No.	Day 2	Day 4	Day 8	Day 15	Day 78
1	x	x	x	x	x
2	x	x	x	x	-
3	x	x	x	x	-
4	x	x	x	x	x
5	x	x	x	x	-

Blood samples were left at room temperature for approximately 1 hour before being centrifuged for approximately 10 min at approximately 3000 rpm (900 g). The resultant serum was separated into uniquely labelled plain polypropylene tubes. The tubes were frozen immediately over dry ice before stored in a freezer set to maintain -20°C.

Thyroid hormone analysis: The serum samples were dispatched on dry ice to WIL Research Laboratories, LLC, 1407 George Road, Ashland OH 44805-8946 and were analysed for Tetraiodothyronine (Thyroxine, T_4), Triiodothyronine (T_3), and Thyroid Stimulating Hormone (TSH).

Terminal procedures*:* Animals were euthanized on their scheduled termination day (treatment days 2, 4, 8 or 15 or recovery day 63) by exposure to a rising concentration of carbon dioxide and had their terminal body recorded followed by severance of major blood vessels. The animals were euthanized in random order throughout the dose groups.

Necropsy: Each animal was subject to a detailed necropsy that consisted of a complete external and internal examination including body orifices (ears, nostrils, mouth, anus and vulva) and cranial, thoracic and abdominal organs and tissues. All gross findings were recorded in descriptive terms, including location(s), size (in mm), shape, colour, consistency and number.

The thyroid glands and liver were removed and weighed from all animals.

Representative samples of the tissues listed in the histopathology table were taken from all animals and fixed in 10% neutral buffered formalin, unless otherwise stated. Carcasses were discarded.

Organ weights: The thyroid gland and liver were weighed.

Microscopic examination: The following tissues processed to paraffin wax block from all animals, sectioned, mounted on glass slides, and stained with haematoxylin and eosin.

Gross lesions including masses (including local draining lymph nodes)

Thyroid gland (including a section of duodenum)
Liver (including a section of duodenum)

Histopathological evaluation of these tissues was then undertaken for all main and recovery study animals.

Liver sampling and analysis: After weighing and sampling for histopathology, the samples of the left, median and caudate lobe liver tissue were processed as follows:

- A section from the left, median and caudate lobes of the liver and a section of duodenum were processed after 36-48 hrs to paraffin wax block. The tissues did not sit in hot wax longer than 2 hours.
- A single tissue section (approximately 4 mm thick) was taken from the left, lateral liver lobe and divided into two and snap frozen in liquid nitrogen. These samples were placed in RNA-ase free tubes and stored deep frozen in a freezer set to maintain -80°C.
- The remaining liver tissue was then sectioned into 4-5 pieces and snap frozen in liquid nitrogen prior to storage in a freezer set to maintain -80°C.

Each liver sample was labelled with the Charles River study number, animal identification, dose group and termination time point. The frozen liver samples were shipped on dry ice to LFR Molecular Sciences, Randalls Road, Leatherhead, Surrey, KT22 7RY, UK.

Each liver sample was separately homogenised and microsomal and cytosolic fractions prepared by differential centrifugation. Aliquots of the whole homogenate, microsomal and cytosolic fractions were stored at -70°C or below. Liver homogenates were assayed for protein content. Liver microsomes were assayed for protein content and UDPGT activity towards thyroxine as substrate.

Microsomal protein content was expressed as per gram of liver. UDPGT activity towards thyroxine as substrate was expressed as per unit of microsomal protein, per gram of liver, per total liver (using the absolute liver weight) and per relative liver weight.

Thyroid sampling and analysis: The thyroid (including parathyroid where possible) and a section of duodenum were processed after 36-48 hours to paraffin wax block. The tissues did not sit in hot wax longer than 2 hours.

The thyroid (containing parathyroid if possible) and duodenum wax blocks were labelled with the Charles River study number, animal identification, dose group and termination time point. Following sectioning for histopathological evaluation, the wax blocks from animals processed following kills on days 2, 4, 8 and 15 as well as those generated after the 63-day recovery period (day 78) were shipped to CXR Biosciences Ltd, 2 James Lindsay Place, Dundee Technopole, Dundee, DD1 5JJ, UK.

Sections of each wax block containing thyroid (including parathyroid where possible) and duodenum were cut 4-6 μm thick, dried overnight at a low temperature and then stained for BrdU cell proliferation evaluation.

Uptake of BrdU was detected utilizing antibodies specific for BrdU followed by a biotinylated secondary antibody and avidin-biotin complex conjugated to horse radish peroxidase. Slides were subject to qualitative assessment prior to being quantitatively evaluated using image analysis software. The results were given as a labelling index, with the replicating cells expressed as a percentage of the total cells.

Statistics:

Liver sampling and analysis: The data were summarised in the form of mean and standard deviations (SDs) of the mean. The liver protein and enzyme activity were tested for normality using the Kolmogorov-Smirnov test and for heterogeneity using Bartlett's test. The data from the control and treated groups were subjected to Dunnett's test in the first instance. In all Dunnett's test comparisons, a probability level of less than 0.05 was accepted to indicate statistical significance.

Data evaluation: The following statistical approaches were used in this study:

- All analyses were two-tailed for significance levels of 5% and 1%.
- All means are presented with standard deviations.
- Body weights, cumulative body weight gain and food consumption were analysed initially by a one-way analysis of variance (ANOVA).
- Organ weights were also analysed by analysis of covariance (ANCOVA) on final body weight (Shirley, 1977). This statistical analysis provided Adjusted Organ Weight values.
- Summary values of organ to body weight ratios are presented but these were not analysed statistically.
- For all parameters evaluated initially by ANOVA or ANCOVA, Dunnett's test was used to compare the control (Group 1) and treated groups, based on the error mean square in the ANOVA or ANCOVA. The Dunnett's test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant.
- Micropathology incidence data were analysed using Fisher's Exact Test. Findings with multiple severities were analysed using a Mann-Whitney U-test.

The reviewer agrees with the statistical procedures used.

RESULTS AND DISCUSSION

Mortality: There were no unscheduled deaths during the observation period.

Clinical observations: There were no treatment-related clinical observations in animals given SYN545192. Observations of bald areas, lesions, scabs or staining to fur were seen in control and treated animals equally. In the positive control animals, there were incidences of rolling or staggering gait, and low body posture were observed during the first 7 days of treatment and on occasion thereafter up to Day 14.

Body weight and weight gain: In SYN545192 groups, body weight gains were decreased compared to controls at doses ≥ 600 ppm in all duration groups. Body weights were decreased compared to controls starting at day three in the 8 and 15 day duration groups at 1200 ppm.

In the positive control groups, body weight gains were decreased in the 2, 4, and 8 day duration groups.

In the recovery group, body weights were decreased until Day 17. Body weights at and after day 21 were not adversely decreased and body weight gains were increased compared to controls over the recovery period. (See Table B.6.8.1/04-2)

Table B.6.8.1/04-2: Intergroup comparison of mean body weights and body weight gains (g) of animals (selected timepoints)[a]

	Dose (ppm)				
	0	**100**	**600**	**1200**	**1200 PB**
Day 2					
Day 0	188 ± 10	188 ± 17	189 ± 17	193 ± 17	189 ± 14
Day 1	193 ± 10	194 ± 18	189 ± 17	184 ± 17	193 ± 14
Days 0 – 1	5 ± 2	5 ± 2	**0 ± 2** (↓100)**	**-9 ± 2** (↓280)**	**4 ± 3 (↓20)**
Day 4					
Day 0	189 ± 17	194 ± 13	181 ± 10	199 ± 12	186 ± 16
Day 3	206 ± 17	211 ± 14	192 ± 10* (↓7)	200 ± 13 (↓3)	200 ± 16 (↓3)
Days 0 – 3	17 ± 3	17 ± 3	**12 ± 3** (↓29)**	**1 ± 6** (↓94)**	**14 ± 5 (↓18)**
Day 8					
Day 0	197 ± 19	186 ± 14	197 ± 15	188 ± 19	189 ± 14 (↓4)
Day 3	215 ± 22	203 ± 15	210 ± 15	**191 ± 18** (↓11)**	204 ± 13 (↓5)
Day 7	238 ± 23	227 ± 15	228 ± 17	**207 ± 20** (↓13)**	225 ± 16 (↓5)
Days 0 – 7	42 ± 6	41 ± 4	**31 ± 5** (↓26)**	**19 ± 5** (↓55)**	**36 ± 6* (↓14)**
Day 15					
Day 0	183 ± 15	186 ± 11	188 ± 15	183 ± 11	187 ± 10
Day 3	201 ± 16	202 ± 12	201 ± 17	**183 ± 11** (↓9)**	202 ± 13
Day 7	224 ± 17	226 ± 11	222 ± 17	**202 ± 12** (↓10)**	226 ± 13
Day 10	239 ± 19	242 ± 12	235 ± 17	**217 ± 14** (↓9)**	245 ± 15
Day 14	256 ± 20	260 ± 11	250 ± 19	**232 ± 15** (↓9)**	260 ± 19
Days 0 – 14	74 ± 10	74 ± 6	**62 ± 8** (↓16)**	**49 ± 9** (↓34)**	72 ± 11
Day 63					
Day 14	254 ± 17			**236 ± 17** (↓7)**	

Day 17	264 ± 19			**249 ± 18* (↓6)**	
Day 21	278 ± 21			266 ± 20	
Days 14 – 77	132 ± 21			148 ± 22	

[a] Data obtained from pages 45 – 50 of the study report
PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, p<0.05
** Statistically significant difference from control group mean, p<0.01

Food consumption: In groups treated with SYN545192, food consumption was decreased on day 1 at doses ≥ 600 ppm and in the positive controls. On day 3, food consumption was decreased at doses ≥ 600 ppm and in the positive controls in the seven day dose group only. At 1200 ppm SYN545192, food consumption was decreased up to day 10. In the recovery group, food consumption was increased on Day 17 and unaffected thereafter. (See Table B.6.8.1/04-3.)

Table B.6.8.1/04-3: Intergroup comparison of mean food consumption (g/animal/day) of animals dosed up to day 15 (selected timepoints)

	Dietary concentration of SYN545192 (ppm)				**PB (ppm)**
	0	**100**	**600**	**1200**	**1200**
Day 2					
Day 0	21.3 ± 0.4	21.6 ± 1.4	21.6 ± 1.1	21.1 ± 1.4	22.4 ± 1.4
Day 1	21.5 ± 1.2	20.7 ± 1.9	**13.7 ± 0.9** (↓36)**	**11.9 ± 3.9** (↓45)**	**17.9 ± 1.6 (↓17)**
Day 4					
Day 0	21.5 ± 1.2	21.8 ± 1.6	21.6 ± .9	22.6 ± 0.8	21.7 ± 1.8
Day 3	22.2 ± 1.5	21.2 ± 1.3	20.4 ± 2.1 (↓8)	**15.3 ± 2.4** (↓31)**	**18.3 ± 2.0* (↓18)**
Day 8					
Day 0	22.1 ± 0.9	21.9 ± 1.5	21.9 ± 0.9	21.6 ± 0.9	22.3 ± 1.4
Day 3	22.9 ± 0.2	20.6 ± 1.3 (↓10)	**19.4 ± 2.0** (↓15)**	**15.5 ± 1.5** (↓32)**	**18.5 ± 1.4** (↓19)**
Day 7	22.6 ± 2.2	23.4 ± 1.9	22.2 ± 1.3	**19.3 ± 0.9* (↓15)**	22.3 ± 1.5
Day 15					
Day 0	21.2 ± 1.1	21.5 ± 1.1	21.1 ± 1.5	21.5 ± 1.3	22.3 ± 1.6
Day 3	20.5 ± 1.2	20.2 ± 1.9	18.6 ± 1.4 (↓9)	**14.5 ± 1.5** (↓29)**	19.3 ± 0.9
Day 7	21.7 ± 1.2	22.6 ± 0.3	22.2 ± 0.6	19.8 ± 1.7** (↓9)	22.8 ± 0.8
Day 10	22.8 ± 1.6	22.1 ± 0.6	21.4 ± 0.9	**20.3 ± 1.6** (↓11)**	23.5 ± 0.9
Day 14	22.5 ± 1.5	23.0 ± 0.9	21.9 ± 2.0	21.7 ± 1.5	24.1 ± 0.8

Day 63					
Day 17	22.2 ± 1.4			**25.0 ± 1.4* (↑13)**	
Day 21	23.8 ± 2.2			25.9 ± 1.9	
Day 24	23.8 ± 2.3			24.8 ± 1.4	
Day 28	21.5 ± 0.9			22.8 ± 2.2	
Day 77	20.9 ± 0.7			19.9 ± 1.3	

[a] Data obtained from pages 51 – 56 of the study report
PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, p<0.05
** Statistically significant difference from control group mean, p<0.01

Dose rates (based on nominal dietary levels of SYN545192) were calculated in terms of mg SYN545192/kg body weight.

Table B.6.8.1/04-4: Mean Dose Received (mg/kg/day)

SYN545192 (ppm)	100	600	1200
Mean achieved dose	9.9	57.7	112.8

Water consumption: Water consumption was monitored visually and not quantitatively. According to the study authors, there was no observable difference in water consumption between the groups.

Thyroid Hormone Analysis: T_3 values were decreased at all time points in the 1200 SYN545192 and positive control groups. At 600 ppm, T_3 was decreased after 1 and 14 days of treatment. In the 100 ppm group, T_3 values were decreased after 14 days of treatment. See Table B.6.8.1/04-5. There were no differences from control in the recovery group.

Table B.6.8.1/04-5: Summary of Total T_3 (ng/dL)[a]

	Dietary concentration of SYN545192 (ppm)				PB (ppm)
Day	**0**	**100**	**600**	**1200**	**1200**
2	138 ± 9.5	126 ± 15.4(↓9)	**107 ± 17.3** (↓22)**	**95 ± 15.2** (↓31)**	**94 ± 12.1** (↓32)**
4	119 ± 15.2	124 ± 21.3	111 ± 21.0(↓7)	**85 ± 16.0** (↓21)**	**93 ± 13.2** (↓21)**
8	118 ± 25.3	121 ± 26.1	107 ± 14.4(↓9)	**103 ± 16.6(↓13)**	**101 ± 25.3(↓14)**
15	119 ± 15.3	**103 ± 19.5* (↓13)**	**92 ± 20.5** (↓23)**	**78 ± 16.3** (↓34)**	**85 ± 20.1** (↓29)**
78	84 ± 16.8	-	-	90 ± 13.2	

[a] Data obtained from pages 192 and 195 of the study report
PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, p<0.05 (Dunnett's test)
** Statistically significant difference from control group mean, p<0.01 (Dunnett's test)

T_4 values were decreased after 1, 3 and 14 days of treatment in 1200 ppm SYN545192 and positive control groups. There was no effect following the recovery period. See Table B.6.8.1/04-6.

Table B.6.8.1/04-6: Summary of Total T_4 (ng/dL)[a]

	Dietary concentration of SYN545192 (ppm)				PB (ppm)
Day	**0**	**100**	**600**	**1200**	**1200**
2	5.8 ± 0.67	6.0 ± 0.50	5.6 ± 0.54	**5.1 ± 0.75** (↓12)**	**4.8 ± 0.60** (↓17)**
4	5.8 ± 0.64	5.7 ± 0.76	5.8 ± 0.97	**4.6 ± 0.82** (↓21)**	**4.6 ± 0.57(↓21)**
8	5.9 ± 0.91	6.4 ± 0.92	6.4 ± 1.20	6.2 ± 0.90	5.9 ± 1.04
15	6.0 ± 0.94	5.8 ± 0.74	5.6 ± 0.49 (↓7)	**5.4 ± 0.93(↓10)**	**5.0 ± 0.39** (↓17)**
78	4.8 ± 0.60	-	-	5.2 ± 0.64	

[a] Data obtained from pages 193 and 195 of the study report
PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, p<0.05 (Dunnett's test)
** Statistically significant difference from control group mean, p<0.01 (Dunnett's test)

TSH values were decreased after one day of treatment at 1200 ppm of SYN545192 and PB. After 14 days of treatment, TSH values were increased at doses ≥ 600 ppm of SYN545192 and in the positive control group.

Table B.6.8.1/04-7: Summary of Total TSH (ng/dL)[a]

	Dietary concentration of SYN545192 (ppm)				PB (ppm)
Day	**0**	**100**	**600**	**1200**	**1200**
2	5.3 ± 1.64	4.9 ± 1.74	4.8 ± 1.61	**4.7 ± 1.47 (↓11)**	**4.7 ± 1.38 (↓11)**
4	3.1 ± 1.52	3.9 ± 1.66	3.7 ± 1.41	3.0 ± 1.75	3.1 ± 1.19
8	5.4 ± 1.40	6.1 ± 3.84	5.7 ± 3.06	4.8 ± 1.90	5.8 ± 2.16
15	4.6 ± 1.96	4.3 ± 1.34	**6.0 ± 3.52 (↑30)**	**7.5* ± 4.41 (↑63)**	**7.8** ± 3.40 (↑70)**
78	4.5 ± 1.66	-	-	4.9 ± 2.36 (↑9)	

[a] Data obtained from pages 194 and 195 of the study report
PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, p<0.05 (Dunnett's test)
** Statistically significant difference from control group mean, p<0.01 (Dunnett's test)

Terminal studies

Scheduled euthanasia: Days 2, 4, 8 and 15: There were no treatment-related gross necropsy findings in the animals treated with SYN545192 or PB.

Scheduled euthanasia: Day 78 (14 days treatment, followed by 63 day recovery period): There were no treatment-related gross necropsy findings in the recovery group.

Organ weights: After one day of treatment, 1200 ppm SYN545192 animals had decreased absolute and relative liver weights compared to the concurrent controls. Animals in the 600 ppm dose group had decreased absolute weights following one day of treatment, but this was considered secondary to body weight decreases.

Absolute liver weights were increased in positive control animals after 3, 7 and 14 days of treatment. Relative liver weights were increased in 1200 ppm SYN545192 and positive control groups after 3, 7 and 14 days of treatment and at 600 ppm after 14 days of treatment.

Absolute and relative thyroid weights were increased compared to controls in 1200 ppm SYN545192 and positive control groups after 14 days of treatment, relative thyroid weights were increased compared to controls in 1200 ppm SYN545192 and positive control groups after 7 days of treatment and absolute thyroid weights were increased compared to controls in positive control animals after 7 days of treatment. (See Tables B.6.8.1/04-08 – 11)

Table B.6.8.1/04-8: Intergroup comparison of liver weight (absolute weights) (g)[a]

Group	Day				
	2	**4**	**8**	**15**	**63**
0 ppm (control)	9.44 ± 0.89	9.19 ± 0.72	10.51 ± 1.24	11.12 ± 1.29	13.09 ± 1.62
100 ppm SYN545192	9.29 ± 0.91	10.10 ± 1.15	10.40 ± 1.06	11.43 ± 1.19	
600 ppm SYN545192	**8.37 ± 0.94* (↓11)**	9.32 ± 0.83	10.89 ± 1.14	11.73 ± 1.60	
1200 ppm SYN545192	**7.73 ± 1.05** (↓18)**	9.98 ± 1.14	10.47 ± 1.39	11.09 ± 0.90	13.12 ± 1.48
1200 ppm PB	9.07 ± 0.94 (↓4)	**10.36 ± 1.03** (↑13)**	**12.45 ± 1.24** (↑18)**	**14.75 ± 1.90** (↑33)**	

PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, $p<0.05$
** Statistically significant difference from control group mean, $p<0.01$

Table B.6.8.1/04-9: Intergroup comparison of liver weight (relative weights) (g)[a]

Group	Day				
	2	**4**	**8**	**15**	**63**
0 ppm (control)	5.002 ± 0.409	4.554 ± 0.271	4.518 ± 0.257	4.342 ± 0.283	3.426 ± 0.298
100 ppm SYN545192	4.934 ± 0.191	4.857 ± 0.390	4.677 ± 0.349	4.473 ± 0.422	
600 ppm SYN545192	4.548 ± 0.351	4.984 ± 0.464	4.894 ± 0.365	**4.779 ± 0.335 (↑10)**	
1200 ppm	**4.290 ± 0.330 (↓14)**	**5.120 ± 0.492 (↑12)**	**5.213 ± 0.514 (↑15)**	**4.931 ± 0.378 (↑14)**	3.445 ± 0.260

SYN545192					
1200 ppm PB	4.790 ± 0.296	**5.296 ± 0.438 (↑16)**	**5.616 ± 0.478 (↑24)**	**5.748 ± 0.590 (↑32)**	

PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, p<0.05
** Statistically significant difference from control group mean, p<0.01

Table B.6.8.1/04-10: Intergroup comparison of thyroid weight (absolute weights) (g)[a]

Group	Day				
	2	**4**	**8**	**15**	**63**
0 ppm (control)	0.0137 ± 0.0023	0.0151 ± 0.0039	0.0148 ± 0.0033	0.0141 ± 0.0026	0.0191 ± 0.0043
100 ppm SYN545192	0.0140 ± 0.0027	0.0146 ± 0.0035	0.0149 ± 0.0022	0.0164 ± 0.0039	
600 ppm SYN545192	0.0116 ± 0.0027	0.0118 ± 0.0030*	0.0151 ± 0.0026	0.0143 ± 0.0028	
1200 ppm SYN545192	0.0133 ± 0.0022	0.0135 ± 0.0031	0.0149 ± 0.0027	**0.0162 ± 0.0037 (↑15)**	0.0180 ± 0.0041
1200 ppm PB	0.0126 ± 0.0022	0.0143 ± 0.0029	**0.0167 ± 0.0031 (↑13)**	**0.0178 ± 0.0030** (↑26)**	

PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, p<0.05
** Statistically significant difference from control group mean, p<0.01

Table B.6.8.1/04-11: Intergroup comparison of thyroid weight (relative weights) (g)[a]

Group	Day				
	2	**4**	**8**	**15**	**63**
0 ppm (control)	0.00727 ± 0.00116	0.00743 ± 0.00162	0.00638 ± 0.00137	0.00552 ± 0.00087	0.00503 ± 0.00124
100 ppm SYN545192	0.00744 ± 0.00154	0.00705 ± 0.00181	0.00671 ± 0.00105	0.00640 ± 0.00146	
600 ppm SYN545192	0.00630 ± 0.00124	0.00631 ± 0.00169	0.00677 ± 0.00110	0.00585 ± 0.00109	
1200 ppm SYN545192	0.00739 ± 0.00103	0.00693 ± 0.00141	**0.00740 ± 0.00106 (↑16)**	**0.00721 ± 0.00162 (↑31)**	0.00476 ± 0.00120
1200 ppm PB	0.00669 ± 0.00134	0.00731 ± 0.00158	**0.00754 ± 0.00143 (↑18)**	**0.00699 ± 0.00133 (↑27)**	

PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, p<0.05
** Statistically significant difference from control group mean, p<0.01

There were no differences in liver or thyroid gland weights in animals that received 1200 ppm SYN545192 following completion of a 63 day recovery period.

Microscopic examination: There were increases in hepatocellular hypertrophy in SYN545192 groups treated with ≥ 600 ppm after 7 and 14 days of treatment. In the positive control animals, hepatocellular hypertrophy was increased after 3 days of treatment. (See Table B.6.8.1/04-12)

Changes to the thyroid were limited to follicular cell hypertrophy in positive control animals following 14 days of treatment. (See Table B.6.8.1/04-13)

There were no changes in recovery animals.

Table B.6.8.1/04-12: Intergroup comparison of microscopic findings in the liver (hepatocyte hypertrophy)

Group	Day				
	2	**4**	**8**	**15**	**63**
0 ppm (control)	0	0	0	0	0
100 ppm SYN545192	0	0	0	0	-
600 ppm SYN545192	0	0	**10****	**13****	-
1200 ppm SYN545192	0	0	**15****	**15****	0
1200 ppm PB	0	**13****	**15****	**15****	-

Values are total incidence out of 15 animals per dose per time interval.
PB = Phenobarbital Sodium Salt
- = Not determined
* Statistically significant difference from control group mean, p<0.05 Fisher's Exact Test
** Statistically significant difference from control group mean, p<0.01 Fisher's Exact Test

Table B.6.8.1/04-13: Intergroup comparison of microscopic findings in the thyroid (follicular cell hypertrophy)

Group	Day				
	2	**4**	**8**	**15**	**63**
0 ppm (control)	0	0	0	0	0
100 ppm SYN545192	0	0	0	0	-
600 ppm SYN545192	0	0	0	0	-
1200 ppm SYN545192	0	0	0	0	0
1200 ppm PB	0	0	0	**8****	-

Values are total incidence out of 15 animals per dose per time interval.
PB = Phenobarbital Sodium Salt
- = Not determined
* Statistically significant difference from control group mean, p<0.05 Fisher's Exact Test
** Statistically significant difference from control group mean, p<0.01 Fisher's Exact Test

Liver biochemistry: Hepatic microsomal UDPglucuronosyltransferase (UDPGT) activity towards thyroxine as substrate was measured as a function of mg protein, g liver, liver weight and body weight. There was a trend towards an initial increase in UDPGT activity after one or three days of treatment, less activity after 7 days of treatment and a final increase after 14 days of treatment. Measured against liver weight and body weight, activity was increased in all treatment groups after 3 days of treatment. Measured against protein and liver, activity was increased at doses ≥ 600 ppm. (See Table B.6.8.1/04-14)

Recovery groups were slightly decreased compared to controls, but not to a statistically significant degree.

In the positive control group, activity was increased after 1 day of treatment and increased at all time points.

Table B.6.8.1/04-14: Intergroup comparison of hepatic microsomal UDPglucuronosyltransferase (UDPGT) activity towards thyroxine as substrate

	Dietary concentration of SYN545192 (ppm)				PB (ppm)
Day	**0**	**100**	**600**	**1200**	**1200**
pmol/min/mg protein					
1	28.1 ± 7.65	28.6 ± 6.19	25.8 ± 4.50	30.0 ± 7.40 (↑7)	**32.8 ± 5.96 (↑17)**
3	28.3 ± 6.50	34.5 ± 7.47	**36.7 ± 6.34* (↑30)**	**42.6 ± 10.32** (↑51)**	**50.1 ± 12.75** (↑77)**
7	33.8 ± 5.12	33.5 ± 7.93	31.4 ± 8.83 (↓7)	**42.2 ± 9.44* (↑25)**	**52.6 ± 10.93** (↑56)**
14	22.1 ± 3.76	20.2 ± 3.93	24.4 ± 5.90	**33.1 ± 4.21** (↑50)**	**43.5 ± 9.09** (↑97)**
Recovery	23.6 ± 8.45	-	-	21.5 ± 7.59 (↓9)	
pmol/min/g liver					
1	971 ± 375.4	1017 ± 294.6	845 ± 190.4 (↓13)	**1081 ± 496.8 (↑11)**	**1191 ± 302.0 (↑23)**
3	968 ± 238.5	1238 ± 295.1	**1333 ± 284.2* (↑38)**	**1695 ± 522.1** (↑75)**	**2067 ± 518.9** (↑114)**
7	1171 ± 232.0	1200 ± 336.7	1224 ± 418.2	**1853 ± 516.2** (↑58)**	**2417 ± 557.0** (↑106)**
14	747 ± 166.9	770 ± 168.1	**1051 ± 329.1** (↑41)**	**1555 ± 262.6** (↑108)**	**2134 ± 549.8** (↑186)**
Recovery	848 ± 345.1			748 ± 243.7 (↓12)	
nmol/min/liver weight					
1	9.24 ± 4.045	9.51 ± 3.127	6.99 ± 1.309 (↓24)	8.15 ± 3.265 (↓12)	**10.81 ± 2.943 (↑17)**
3	8.97 ± 2.531	**12.42 ± 2.905** (↑39)**	**12.43 ± 3.116** (↑39)**	**17.06 ± 6.467** (↑90)**	**21.41 ± 5.741** (↑139)**
7	12.24 ± 2.389	12.66 ± 4.405	13.21 ± 4.685 (↑8)	**19.39 ± 4.681** (↑58)**	**29.80 ± 6.390** (↑143)**

14	8.19 ± 1.431	8.80 ± 2.165 (↑7)	**12.16 ± 3.584** (↑48)**	**17.22 ± 3.046** (↑110)**	**31.43 ± 9.600** (↑284)**
Recovery	11.02 ± 4.440			9.80 ± 3.101 (↓11)	
nmol/min/kg body weight					
1	48.85 ± 20.840	50.33 ± 15.040	38.07 ± 7.256 (↓22)	45.43 ± 18.282 (↓7)	**57.20 ± 15.410 (↑17)**
3	44.03 ± 10.343	**60.05 ± 14.735** (↑36)**	**66.52 ± 17.620** (↑51)**	**86.89 ± 29.756** (↑97)**	**110.53 ± 34.358** (↑151)**
7	52.59 ± 8.958	56.62 ± 18.282	**59.69 ± 21.090 (↑14)**	**96.99 ± 32.342** (↑84)**	**134.92 ± 29.614** (↑157)**
14	32.27 ± 6.801	34.47 ± 8.326	**49.82 ± 14.596** (↑54)**	**76.43 ± 12.507** (↑137)**	**122.44 ± 34.067** (↑279)**
Recovery	28.48 ± 10.419			25.49 ± 7.461 (↓10)	

[a] Data obtained from pages 434, 436, 438, 440
PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, p<0.05 (Dunnett's test)
** Statistically significant difference from control group mean, p<0.01 (Dunnett's test)

Conversely, with hepatic microsomal protein content, as time increased there was a consistent increase in severity and a decrease in doses at which there was a treatment-related increase in protein content. After 14 days of treatment, hepatic microsomal protein content was increased in all treated groups. (See Table B.6.8.1/04-15)

In the recovery group, there was no effect on hepatic microsomal protein content.

Table B.6.8.1/04-15: Intergroup comparison of hepatic microsomal protein content

	Dietary concentration of SYN545192 (ppm)				**PB (ppm)**
Day	**0**	**100**	**600**	**1200**	**1200**
1	33.8 ± 4.85	35.1 ± 4.22	32.6 ± 3.39	35.0 ± 9.58	35.9 ± 3.68
3	34.2 ± 2.71	35.8 ± 1.96 (↑5)	36.2 ± 3.09 (↑6)	**39.4 ± 4.12** (↑15)**	**41.4 ± 2.95** (↑21)**
7	34.5 ± 3.26	35.6 ± 2.84 (↑3)	**38.6 ± 2.77** (↑12)**	**43.7 ± 5.12** (↑27)**	**45.8 ± 2.73** (↑33)**
14	33.7 ± 2.83	**38.1 ± 3.87* (↑13)**	**42.7 ± 4.87** (↑27)**	**47.0 ± 5.33** (↑39)**	**48.9 ± 5.60** (↑45)**
Recovery	35.5 ± 3.57	-	-	35.0 ± 2.73 (↓1)	-

[a] Data obtained from pages 433, 435, 437, 439
PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, p<0.05 (Dunnett's test)
** Statistically significant difference from control group mean, p<0.01 (Dunnett's test)

Cell proliferation measurements: Thyroid S-phase labelling was inconclusive in the first 8 days of the study as there was a statistically significant increase in labelling in the low-dose animals and no dose-response. By day 15 sampling, S-phase labelling was increased in 1200 ppm SYN545192 and positive controls and the increases at day 15 were correlated with

thyroid hormone, organ weight and histological changes. There were no changes to S-phase labelling in the recovery animals. (See Table B.6.8.1/04-16)

Table B.6.8.1/04-16: Intergroup comparison of thyroid S-phase labelling[a]

	Dietary concentration of SYN545192 (ppm)				PB (ppm)
Day	**0**	**100**	**600**	**1200**	**1200**
1	1.29 ± 0.29	2.21 ± 0.29** (↑71)	1.81 ± 0.62 (↑40)	1.61 ± 0.54 (↑25)	2.40 ± 0.93 (↑86)
3	1.03 ± 0.39	2.25 ± 0.46** (↑118)	2.78 ± 0.69** (↑170)	1.34 ± 0.50 (↑30)	1.56 ± 0.87* (↑51)
7	0.69 ± 0.26	2.47 ± 0.62** (↑258)	0.87 ± 0.32 (↑26)	1.88 ± 0.84** (↑172)	1.62 ± 0.60** (↑135)
14	0.46 ± 0.21	0.70 ± 0.35	0.60 ± 0.19 (↑30)	**2.18 ± 0.69** (↑374)**	**2.15 ± 0.80** (↑367)**
Recovery	0.36 ± 0.11	-	-	0.30 ± 0.09 (↓17)	-

[a] Data obtained from page 467 of the study report
PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, $p<0.05$ (Dunnett's test)
** Statistically significant difference from control group mean, $p<0.01$ (Dunnett's test)

Investigators' Conclusion: This study has demonstrated that dietary treatment of male rats with SYN545192 results in increased hepatic UDPGT activity, hepatocellular centrilobular hypertrophy, increased liver weight, decreased serum T_3 and T_4, and increased serum TSH associated with increased thyroid follicular cell replicative DNA synthesis and thyroid weight. These effects can be used as part of a weight-of-the-evidence approach to explain the mode of action for the increased incidence of thyroid follicular cell adenomas observed in male Han Wistar rats in a 2 year carcinogenicity study (***Charles River Study No. 459580***).

Reviewer's Conclusions:

The reviewer is in agreement with the investigators' conclusions that SYN545192 has similar effects on the thyroid and liver as the positive control, phenobarbital. At comparable doses, the effects of phenobarbital and SYN545192 were within the same magnitude. At the tumourigenic dose of 600 ppm, several of the parameters showed a weak response.

Reference:

Mackay, C (2012): SYN545192 – 104 Week Rat Dietary Carcinogenicity Study with a Combined 52 Week Toxicity Study. Charles River Study No. 459580. Syngenta File No. SYN545192_10183).

Report:	IIA 5.5.4/02. Lake B, 2012b. SYN545192: Effect on Hepatic UDPglucuronosyltransferase activity towards thyroxine as substrate after dietary administration for up to 28 days to male rats. Leatherhead Food Research (LFR), Molecular Sciences Department, Randalls Road, Leatherhead, Surrey, KT22 7RY, United Kingdom. Laboratory Report No. 5496/1/2/2012, 20 February 2012. Unpublished (Syngenta File No.SYN545192_10192). EPA MRID No. 48604554

Guidelines: There is no applicable test guideline for an investigative toxicity study of this type.

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

The aim of this study was to evaluate the effect of treatment with SYN545192 on hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine (tetraiodothyronine; T4) as substrate in the livers of male rats. Male rats were given diets containing 0 (control), 100, 750 and 1500 ppm SYN545192 for periods of 3, 7, 14 and 28 days. In addition, the effect of treatment of male rats with 0 (control) and 1200 ppm phenobarbital for 7 days on hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate was also determined. Phenobarbital is a known inducer of hepatic cytochrome P450 (CYP) forms in the rat and is also known to induce microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate and was therefore used as a positive control in this study. Liver microsomes from control, SYN545192- and phenobarbital-treated rats were assayed for protein content and UDPglucuronosyltransferase activity towards thyroxine as substrate. Enzyme activity was expressed as specific activity (i.e. per unit of microsomal protein), per gram of liver, per total liver and per relative liver weight.

There were no effects on hepatic microsomal protein content or UDPglucuronosyltransferase activity towards thyroxine as substrate at 100 ppm. Effects on hepatic microsomal protein content only occurred after 7 days of treatment at doses ≥ 750 ppm. UDPglucuronosyltransferase activity towards thyroxine as substrate was increased as expressed as specific activity, per gram of liver, per total liver and per relative liver weight after 7 and 28 days of treatment at doses ≥ 750 ppm. After 3 days of treatment, UDPglucuronosyltransferase activity towards thyroxine as substrate was increased as expressed as specific activity, per total liver and per relative liver weight at doses ≥750 ppm. After 14 days of treatment, UDPglucuronosyltransferase activity towards thyroxine as substrate was increased as expressed per relative liver weight only at 1500 ppm.

The treatment of male rats with 1200 ppm phenobarbital for 7 days significantly increased hepatic microsomal protein content confirming the responsiveness of the test system towards this positive control. In the phenobarbital-treated animals, hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate expressed as specific activity, per gram of liver, per total liver and per relative liver weight was significantly increased.

Based on the results of this study, SYN545192 at doses of 750 ppm and greater is an inducer of hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate in male rats.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545192
Description:	Technical material, white solid
Lot/Batch number:	TE-6341
Purity:	98.3% a.i
CAS#:	Not reported
Stability of test compound:	Reanalysis date end August 2010

Descriptive information on the test material (SYN545192) was provided in the report for a 28-day rat study (Robertson B, 2010a), from which the SYN545192-treated livers used in this study were derived.

Vehicle / positive control: Control diet / phenobarbital.

Positive Control Material:	Phenobarbital sodium salt
Description:	White powder
Lot/Batch number:	031M1417V
Purity:	100%
CAS#:	Not reported
Stability of test compound:	Reanalysis date 31 January 2015

Descriptive information on the positive control material (phenobarbital) was provided in the report for a 28-day rat study (Donald, 2012), from which the phenobarbital-treated livers used in this study were derived.

Test Animals:	
Species	Rats
Strain	Wistar Han
Age/weight at dosing	262-302 g
Source	Charles River UK Ltd., Margate, Kent, CT9 4LT, UK.
Housing	Housing, diet, water and environmental conditions reported in Donald, 2012
Diet	
Water	
Environmental conditions	

Study Design and Methods:

Experimental dates: Start: 29 November 2011 End: 04 January 2012

Animal dosing / test system: The test system consisted of frozen samples of rat liver collected from two studies. In one study, male rats were given diets containing 0 (control), 100, 750 and 1500 ppm SYN545192 for periods of 3, 7, 14 and 28 days (Robertson, 2010b). In the second study, male rats were given diets containing 0 (control) and 1200 ppm phenobarbital, which served as a positive control, for 7 days (Donald, 2012). Liver samples from control, SYN545192-

treated and phenobarbital-treated rats were collected during necropsy and stored at or below -70ºC.

Details of the liver samples collected from these two studies are as follows:

Treatment	Study number	Animal numbers			
		3 days	7 days	14 days	28 days
Control	459287	5	5	5	5
SYN545192 100 ppm		5	5	5	5
SYN545192 750 ppm		5	5	5	5
SYN545192 1500 ppm		5	5	5	5
Control	521213		5		
Phenobarbital 1200 ppm			5		

Preparation of liver fractions: Each liver sample was thawed and weighed. Whole homogenates of the individual liver samples were prepared in 0.154 M KCl containing 50 mM Tris-HCl, pH 7.4 and centrifuged at 10,000 g average for 20 minutes to obtain the postmitochondrial supernatants which were subsequently centrifuged at 105,000 g average for 60 minutes to separate the microsomal fraction from the cytosol. The microsomal fraction of each animal was resuspended in fresh homogenising medium. Aliquots of liver whole homogenate, microsomal and cytosolic fractions from each animal were stored at -70°C or below.

Assay of protein content: Liver whole homogenate and microsomal protein content were determined by the general procedure of ***Lowry et al. (1951***), as described by ***Lake (1987),*** employing bovine serum albumin as standard.

Assay of UDPglucuronosyltransferase activity: Hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate was determined in incubation mixtures containing 10 µM thyroxine and 5 mM UDPGA (***Finch et al, 2006***). The formation of thyroxine glucuronide was quantified by ultra performance liquid chromatography-mass spectrometry-mass spectrometry (UPLC-MS-MS). The formation of thyroxine glucuronide in rat liver microsome incubations was calibrated by determining the formation of thyroxine glucuronide (peak area) compared to the loss of thyroxine substrate in incubations with expressed human UGT1A8.

Statistics: Data were summarised in the form of mean and standard deviations (SDs) of the mean. Hepatic microsomal protein content and UDPglucuronosyltransferase enzyme activity data were tested for normality using the Kolmogorov-Smirnov test (level of significance determined to be at $p<0.05$) and heterogeneity using Bartlett's test (level of significance $p<0.01$). Control and SYN545192 treated groups were subjected to a one-way analysis of variance and comparisons between control and SYN545192 treated groups were made using two-sided Dunnett's tests. The data from the control and phenobarbital treated groups were subjected to a one-way analysis of variance and comparisons between control and phenobarbital treated groups were made using two-sided t-tests. In all Dunnett's test and t-test comparisons a probability level of $p<0.05$ was taken to indicate statistical significance.

RESULTS AND DISCUSSION

Treatment with SYN545192 for 3 days: After three days of treatment, there was no effect on hepatic microsomal protein content at any dose.

At doses ≥ 750 ppm, hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate expressed as specific activity, per total liver and per relative liver weight was increased. There was no effect on hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate expressed per gram of liver. (See Table B.6.8.1/02-1)

Treatment with SYN545192 for 7 days: After seven days of treatment, hepatic microsomal protein content was increased at doses ≥ 750 ppm. (See Table B.6.8.1/02-1)

There was no effect on hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate at 100 ppm. Expressed as specific activity, per gram of liver, per total liver and per relative liver weight, hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate was increased at doses ≥ 750 ppm.

Treatment with SYN545192 for 14 days: After 14 days of treatment, there was no effect on hepatic microsomal protein content at any dose. (See Table B.6.8.1/02-1)

Hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate was increased to a statistically significant degree expressed per liver weight and per relative liver weight at doses ≥ 750 ppm and per gram liver at 750 ppm. Other than per relative liver weight there was no dose response.

Treatment with SYN545192 for 28 days: After 28 days of treatment, there was no effect on hepatic microsomal protein content at any dose. (See Table B.6.8.1/02-1)

Hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate was increased to a statistically significant degree only expressed per relative liver weight at 1500 ppm. However, there was a biologically significant increase in activity towards thyroxine as substrate expressed as specific activity, per gram of liver, per total liver and per relative liver weight at doses ≥ 750 ppm.

Treatment with phenobarbital for 7 days: The positive control treatments confirmed the validity of the test system. After treatment for 7 days with 1200 ppm phenobarbital, hepatic microsomal protein content was increased and hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate was increased as expressed as specific activity, per gram of liver, per total liver and per relative liver weight. (See Table B.6.8.1/02-1)

Assays with reference items: The potential responsiveness of hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate determined in this study to an inducer of hepatic CYP forms was assessed with liver microsomes from vehicle treated (corn oil) and β-naphthoflavone (BNF) treated male Sprague-Dawley rats. Microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate was induced to 1529% of

control by BNF treatment, enzyme activity being 44.4 and 678.9 pmol/min/mg protein in corn oil and BNF-treated rat liver microsomes, respectively.

Table B.6.8.1/02-1: Summary of the effect of treatment[a]

Treatment / days of dosing		Hepatic Microsomal Protein Content	UDPglucuronosyltransferase activity (substrate thyroxine)			
		(mg/g liver)	pmol/min/mg protein	pmol/min/g liver	nmol/min/liver weight	nmol/min/liver weight/kg body weight
Control	3	21.8 ± 3.01	23.4 ± 4.10	516 ± 158.2	4.47 ± 1.373	21.31 ± 6.276
100 ppm		25.3 ± 1.69	23.0 ± 5.09 (98)	587 ± 160.0 (114)	5.84 ± 2.037 (131)	27.69 ± 9.014 (130)
750 ppm		24.6 ± 3.95	**32.0 ± 6.65 (137)***	793 ± 248.1 (154)	**8.44 ± 2.669 (189)***	**38.95 ± 13.762 (183)***
1500 ppm		24.9 ± 3.03	**32.3 ± 4.07 (138)***	814 ± 201.1 (158)	**8.12 ± 1.305 (182)***	**39.31 ± 7.374 (184)***
Control	7	21.8 ± 3.12	18.2 ± 4.35	395 ± 114.0	4.02 ± 1.148	16.71 ± 4.692
100 ppm		21.4 ± 0.79	19.5 ± 5.68 (107)	414 ± 113.3 (105)	4.45 ± 0.850 (111)	19.05 ± 4.276 (114)
750 ppm		**26.6 ± 3.57 (122)***	**27.6 ± 4.91 (152)***	**724 ± 81.1 (183)****	**8.01 ± 1.117 (199)****	**33.55 ± 3.836 (201)****
1500 ppm		**29.7 ± 2.96 (136)****	**34.6 ± 3.83 (190)****	**1025 ± 122.5 (259)****	**11.94 ± 2.603 (297)****	**52.74 ± 7.930 (316)****
Control	14	22.4 ± 3.12	21.8 ± 8.22	497 ± 231.3	4.98 ± 2.245	19.91 ± 9.057
100 ppm		25.8 ± 2.78	26.8 ± 5.59 (123)	694 ± 169.5 (140)	7.45 ± 1.513 (150)	27.64 ± 5.400 (139)
750 ppm		28.4 ± 3.61	29.8 ± 2.30 (137)	849 ± 141.9 (171)*	10.49 ± 2.370 (211)**	39.70 ± 6.356 (199)**
1500 ppm		26.0 ± 6.06	29.2 ± 5.82 (134)	767 ± 266.8 (154)	10.01 ± 3.616 (201)*	**40.04 ± 13.314 (201)****
Control	28	25.6 ± 7.34	18.0 ± 7.57	481 ± 249.4	5.25 ± 2.463	17.65 ± 8.565
100 ppm		21.2 ± 2.14	21.4 ± 6.93 (119)	464 ± 190.6 (96)	5.72 ± 1.596 (109)	19.18 ± 6.064 (109)
750 ppm		25.5 ± 4.33	**26.8 ± 5.93 (149)**	**698 ± 255.8 (145)**	**8.12 ± 2.987 (155)**	**28.95 ± 11.484 (164)**
1500 ppm		27.8 ± 6.50	**31.3 ± 11.77 (174)**	**930 ± 614.7 (193)**	**12.02 ± 7.360 (229)**	**42.95 ± 23.388 (243)***
Control	7	26.6 ± 4.14	29.9 ± 3.83	801 ± 187.3	10.11 ± 2.528	35.64 ± 8.421
Phenobarbital 1200 ppm		**39.8 ± 4.80****	**53.0 ± 4.67 (177)****	**2125 ± 421.2 (265)****	**31.51 ± 4.915 (312)****	**118.76 ± 20.078 (333)****
values in parentheses are % of control levels, * p<0.05, ** p<0.01 by Dunnett's test (SYN545192) or t-test (phenobarbital)						
Bolded values determined to be treatment related						
[a] Data obtained from pages 19 – 28 of the study report						

INVESTIGATOR'S CONCLUSION: SYN545192 at doses of 750 ppm and greater is an inducer of hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate in male rats.

REVIEWER'S CONCLUSIONS: The reviewer agrees with the investigators that SYN545192 at doses ≥ 750 ppm induced hepatic microsomal UDPglucuronosyltransferase activity towards thyroxine as substrate in male rats.

REFERENCES:

Donald L, 2012. Bicyclopyrone - 28 Day Dietary Thyroid Mode of Action Study in Rats. Charles River, Tranent, Edinburgh, UK. CRL Report No. 32992. Study No. 521213. Unpublished (Syngenta File No. NOA449280_11266). US EPA MRID No. 47841990.

Finch JM., Osimitz TG, Gabriel KL, Martin T, Henderson WJ, Capen CC, Butler WH, Lake BG, 2006. A mode of action for induction of thyroid gland tumors by Pyrethrins in the rat. *Toxicol. Appl. Pharmacol.* 214, 253-262.

Lake BG, 1987. Preparation and characterisation of microsomal fractions for studies of xenobiotic metabolism, In: Snell K, Mullock B (Eds.), Biochemical Toxicology: A Practical Approach, IRL Press, Oxford, pp. 183−215.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. 193, 265-275.

Robertson B, 2010b. SYN545192: Investigative 28 day dietary study in rats with interim kills. Charles River Study No. 459287. Syngenta Task No. T004190-08. Unpublished. (Syngenta File No. SYN545192_10048). US EPA MRID No. 48604588.

Report:	IIA 5.5.4/01. Lake B, 2012a. SYN545192 - Effect on rat thyroid peroxidase activity in vitro. Leatherhead Food Research (LFR), Molecular Sciences Department, Randalls Road, Leatherhead, Surrey, KT22 7RY, United Kingdom. Laboratory Report No. 5497/1/1/2012, 06 February 2012. Unpublished (Syngenta File No. SYN545192_10190.). EPA MRID No. 48604555

Guidelines

There is no applicable test guideline for an investigative toxicity study of this type.

GLP: This study was not conducted according to Good Laboratory Practice Standards as defined by OECD. No claim of GLP compliance was made for this study.

EXECUTIVE SUMMARY

The aim of this study was to evaluate the effect of SYN545192 (purity 97%) on rat thyroid peroxidase activity *in vitro* at dose levels of 0 (control), 0.01, 0.1, 1 and 10 µM. A pooled thyroid gland microsomal preparation from five rats was assayed for thyroid peroxidase activity by determining the monoiodination of L-tyrosine. As a positive control, the effect of 6-propyl-2-thiouracil (PTU; 10 µM) on rat thyroid peroxidase activity was also determined.

Treatment with SYN545192 had no significant effect on rat thyroid peroxidase activity at any concentration tested. Treatment with PTU resulted in a 100% inhibition of thyroid peroxidase activity and validated the test system.

Based on the results of this study, SYN545192 is not an inhibitor of rat thyroid peroxidase activity *in vitro*.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545192
Description:	Beige powder
Lot/Batch number:	SMU9BP005
Purity:	97%
CAS#:	1072957-71-1
Stability of test compound:	Stable until end February 2013 if stored at <30°C

Vehicle / positive control: Dimethyl sulphoxide (DMSO) / 10 µM 6-propyl-2-thiouracil (PTU).

Test Animals:

Species	Rats
Strain	Wistar Han
Age/weight at dosing	262-302 g
Source	Charles River UK Ltd., Margate, Kent, CT9 4LT, UK.
Housing	Not reported
Diet	*Ad libitum*
Water	*Ad libitum*
Environmental conditions	Not reported

Study Design and Methods:

Experimental dates: Start: 25 November 2011 End: 07 December 2011

Preparation of thyroid gland microsomes: Five male Wistar Han rats were killed by carbon dioxide asphyxiation and the thyroid gland attached to the part of the trachea was immediately removed and snap frozen in dry ice and stored at -70°C or below until required. The trachea/thyroid glands were thawed and each thyroid gland dissected from the attached trachea. A whole homogenate of the pooled thyroid glands from the 5 rats was prepared in 0.154 M KCl containing 50 mM Tris-HCl, pH 7.4. The thyroid gland whole homogenate was centrifuged at 9,000 g average for 20 minutes to obtain the post-mitochondrial supernatant which was subsequently centrifuged at 105,000 g average for 60 minutes to separate the microsomal fraction from the cytosol. The pooled thyroid gland microsomal fraction was resuspended in fresh homogenising medium. Aliquots of the pooled thyroid gland microsomal fraction were stored at -70°C or below and were thawed once only for the determination of thyroid peroxidase activity.

Assay of Protein Content: Thyroid gland microsomal protein content was determined by the general procedure of ***Lowry et al. (1951)***, employing bovine serum albumin as standard. The microsomal protein content of the pooled thyroid gland preparation was calculated to be 14.9 mg protein/g tissue.

Assay of Thyroid Peroxidase Activity: Thyroid peroxidase activity was assayed by determining the monoiodination of L-tyrosine. Incubations contained 150 µM L-tyrosine, 150 µM potassium iodide, 44 µg thyroid microsomal protein, either SYN454192 or PTU dissolved in dimethyl sulphoxide (2.5 µL/incubation) and 0.1 M phosphate buffer pH 7.4 in a total volume of 0.25 mL. After a 10 minute pre-incubation in a shaking water bath at 37°C, the reaction was initiated by the addition of 200 µM hydrogen peroxide. Blank incubations (to correct for non enzymatic formation of 3-iodo-L-tyrosine) contained all additions except for thyroid gland microsomes. After a 10 minute incubation in a shaking water bath at 37°C the reaction was terminated and levels of 3-iodo-L-tyrosine in deproteinised supernatants determined by ultra performance liquid chromatography-mass spectrometry-mass spectrometry (UPLC-MS-MS). Under these conditions the rate of formation of 3-iodo-L-tyrosine was linear with respect to both incubation time and protein concentration and the formation of 3,5-diiodo-L-tyrosine in control incubations was <2.5% of the formation of 3-iodo-L-tyrosine.

Statistics: Data were summarised in the form of mean and standard deviations (SDs) of the mean. Enzyme activity data were tested for normality using the Kolmogorov-Smirnov test (level of significance determined to be at $p<0.10$) and heterogeneity using Bartlett's test (level of significance $p<0.01$). Where transformations were required all were successful. Control and SYN545192 treated groups and control and PTU treated groups were subjected to a one-way analysis of variance. Comparisons between control and SYN545192 treated groups were made using two-sided Dunnett's tests and between control and PTU treated groups were made using a t-test. In all Dunnett's test and t-test comparisons a probability level of $p<0.05$ was taken to indicate statistical significance.

RESULTS AND DISCUSSION

There was no statistically or biologically significant effect on thyroid peroxidase activity at any dose *in vitro*. Treatment with the positive control chemical, PTU, resulted in complete cessation of activity and confirmed the validity of the test system.

Table B.6.8.1/01-1: Effect of SYN545192 and PTU on rat thyroid peroxidise activity

Addition (a)	Thyroid peroxidase activity (nmol/min/mg protein) (b)	Percentage of control values
Control (DMSO only)	1.03 ± 0.057	100
SYN545192 0.01 µM	1.08 ± 0.092	105
SYN545192 0.1 µM	1.08 ± 0.106	105
SYN545192 1 µM	0.92 ± 0.046	89
SYN545192 10 µM	1.03 ± 0.111	100
PTU 10 µM	0.0 ± 0.00***	0

(a) SYN545192 and PTU were added in DMSO (2.5 µL/incubation).
(b) results are presented as mean±SD for triplicate incubations
*** significantly different from control $p<0.001$

INVESTIGATORS' CONCLUSION: SYN545192 is not an inhibitor of rat thyroid peroxidase activity *in vitro*.

REVIEWER CONCLUSIONS: The reviewer agrees with the investgators that SYN545192 is not an inhibitor of rat thyroid peroxidase activity *in vitro*.

Report:	IIA 5.5.4/03. Robertson B, 2012a. SYN545192: A histological extension study of male thyroid tissue from rat toxicity study (Charles River Study No. 459287). Charles River, Tranent, Edinburgh, EH33 2NE, UK. Laboratory Report No. 33043, 19 June 2012. Unpublished. (Syngenta File No. SYN545192_10213). EPA MRID No. 48604556

Guidelines: There is no applicable test guideline for an investigative toxicity study of this type.

GLP: Signed and dated GLP and Quality Assurance statements were provided.

STUDY TYPE: A Histological Extension Study of Male Thyroid Tissue from Rat Toxicity Study.

No applicable guidelines.

TEST MATERIAL (PURITY): SYN545192 (Purity 98.3%)

SYNONYMS: 3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (9-dichloromethen-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide; Product Code: CSCD064398

CITATION: Robertson B, 2012a. SYN545192: A histological extension study of male thyroid tissue from rat toxicity study (Charles River Study No. 459287). Charles River, Tranent, Edinburgh, EH33 2NE, UK. Laboratory Report No. 33043, 19 June 2012. Unpublished. (Syngenta File No. SYN545192_10213)

SPONSOR: Syngenta Ltd., Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, United Kingdom.

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

In a subchronic toxicity study, SYN545192 (purity 98.3%) was administered orally in the diet to groups of 25 male Han Wistar Crl: WI(Han) rats at dose levels of 100, 750 or 1500 ppm daily over a period up to 28 consecutive days. A concurrent control group received the vehicle alone (Rat and Mouse (modified) No.1 Diet SQC Expanded (Fine Ground)). Animals were terminated after 3, 4, 8, 15 or 29 days of treatment, respectively (i.e. after 2, 3, 7, 14 or 28 full days of dietary exposure).

There was an increase in diffuse follicular cell hypertrophy following 7, 14 and 28 days of treatment at 1500 ppm and following 28 days of treatment at 750 ppm.

Dietary administration of SYN545192 resulted in minimal follicular cell hypertrophy at 1500 ppm over 8, 15 and 29 days and at 750 ppm over 29 days of administration.

MATERIALS AND METHODS

Materials:

Test Material: SYN545192
Description: Technical material, white solid
Lot/Batch number: TE-6341
Purity: 98.3% a.i
CAS#: Not reported
Stability of test compound: Reanalysis date end August 2010

Descriptive information on the test material (SYN545192) was provided in the report for a 28-day rat study (Robertson B, 2010a), from which the SYN545192-treated livers used in this study were derived.

Vehicle and/or positive control: Rat and Mouse (modified) No.1 Diet SQC Expanded (Fine Ground)

Test Animals:
Species Rat
Strain Han Wistar Crl: WI(Han)

No further details reported.

Study Design and Methods:

Experimental dates: Start: 21 November 2011 End: 14 December 2011

Animal assignment: The groups of Charles River Study No. 459287, from which the thyroids were examined, were constructed as follows.

Table 5.5.4/03-1: Study design

Group	Dietary concentration SYN545192 (ppm)	Male animal numbers				
		Time points (day)				
		3	4	8	15	29
1	0 (Control)	5	5	5	5	5
2	100	5	5	5	5	5
3	750	5	5	5	5	5
4	1500	5	5	5	5	5

Histology and histopathology: Male thyroid tissue was previously collected and embedded in paraffin wax blocks. Sections were subsequently mounted on glass slides, and stained with haematoxylin and eosin.

Histopathological evaluation was performed by a board certified veterinary pathologist. A staged reading/evaluation of the tissues was performed therefore slides were only read from lower dose groups if findings existed in the dose group above it. Thyroids from all animals were evaluated.

An internal peer review was undertaken by an appropriately qualified and experienced veterinary pathologist who validated the conclusions of the Study Pathologist by independently assessing the microscope slides.

An external peer review was conducted by: Jayne Wright, Syngenta Ltd., Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, United Kingdom.

Following sampling of the liver for histopathology and possible future immunohistochemistry in Charles River Study No. 459287, the remainder of the liver tissues were snap frozen in liquid nitrogen in RNA-ase free tubes and stored at -70°C for possible future analysis.

Statistical analysis: Micropathology incidence data were analysed using Fisher's Exact Test.

RESULTS AND DISCUSSION

There were no histological changes following 3 days of treatment. After 7, 14 and 28 days of treatment, there was an increase in follicular cell hypertrophy at 1500 ppm. After 28 days of treatment, there was an increase in follicular cell hypertrophy at 750 ppm.

Table B.6.8.1/03-2: Summary of histopathological findings in the thyroid[a]

Dose	Thyroid follicular cell hypertrophy, diffuse, minimal			
	Day 4	Day 8	Day 15	Day 29
0 ppm	0/5	0/5	0/5	0/4
100 ppm	0/5	0/5	0/5	0/5
750 ppm	0/5	0/5	0/5	**2/4**
1500 ppm	0/5	**1/5**	**2/5**	**1/5**

[a] Data obtained from pages 18 – 21 of the study report.

INVESTIGATOR'S CONCLUSION: Dietary administration of SYN545192 resulted in minimal follicular cell hypertrophy at 1500 ppm over 8, 15 and 29 days and at 750 ppm over 29 days of administration.

REVIEWER CONCLUSIONS: The reviewer agrees that there was an increase in follicular cell hypertrophy in the thyroid following 7, 14 and 28 days of treatment at 1500 ppm and 28 days of treatment at 750 ppm.

Reference: Robertson B, 2010b. SYN545192: Investigative 28 day dietary study in rats with interim kills. Charles River Study No. 459287. Syngenta Task No. T004190-08

B.6.1.2

Report:	IIA 5.1.2/0.1 Tomlinson J and Hutton E, 2012. SYN545192 – Pharmacokinetics of Total radioactivity in the Rat following Intravenous and Oral Administration of [^{14}C]-SYN545192. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Charles River Report No. 32748. Issue date 11 May 2012. Unpublished (Syngenta File No. SYN545192_10207). EPA MRID No. 48604579

Guidelines: Metabolism – rat; OECD 417 (2010); EPA OPPTS 870.7485 (1998); 87/302/EEC (1987), B36, 94/79/EC (1994), JMAFF 12 Nohsan No 8147(2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

This study was designed to examine the kinetics of total radioactivity in the blood following oral and intravenous (i.v.) administration of [pyrazole-5-^{14}C]-SYN545192 to rats. The oral bioavailability was determined by comparing the dose-normalised exposure following intravenous and oral administration of [^{14}C]-SYN545192.

After a 0.5 mg [pyrazole-5-^{14}C]-SYN545192/kg i.v. dose resulted in unreliable measurements of bioavailability, groups of 4 Han Wistar rats/sex were dosed at 1 mg/kg bw oral or 0.25 mg/kg bw per day i.v. [pyrazole-5-^{14}C]-SYN545192. Blood was collected via tail venipuncture at 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 96 hours for animals in the oral dose groups and 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 hours for animals in the i.v. dose groups.

In orally dosed animals, male blood concentrations peaked at 4 hours and blood concentrations peaked at 2 hours in females. In i.v. dosed animals, both males and females had peak blood concentrations at 5 minutes.

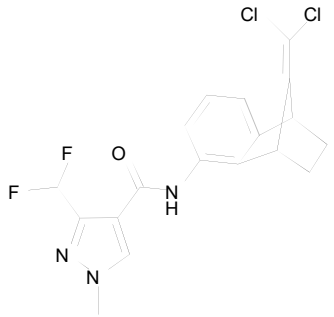
In the pharmacokinetic parameters, the half-lives were comparable regardless of route of exposure at 47.97-53.57 h in males and 25.40-30.86 h in females in oral and i.v. doses, respectively. C_{max}/C_0 values were roughly 2X higher in oral doses compared to i.v. doses, though the absolute dose was 4X higher. Area under the curve values (0 – t and 0 - ∞) were 5.8X higher in male oral doses than i.v. doses and 4.3 and 3.2X higher in female oral doses than i.v. doses (0 – t and 0 - ∞, respectively). Extrapolated AUC (%) values were comparable in males (23% in oral and i.v. doses), though higher than values in females where extrapolated AUC values were 7.3% in oral female doses and 11% in i.v. female doses.

Oral bioavailability was 129% in males and 98.7% in females, indicating that the oral bioavailability was essentially complete.

MATERIALS AND METHODS

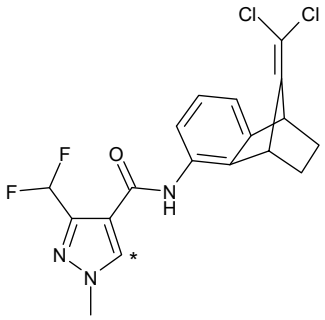
Materials:

Unlabelled Test Material: SYN545192
Purity: 97%
Source: Syngenta Crop Protection Inc.
Lot/Batch number: SMU9BP005
Structure:



Unlabelled test item was used in the oral dose preparation to dilute the specific activity of the radiolabelled test item

Radiolabelled Test Material [Pyrazole-5-^{14}C]-SYN545192
Specific activity: Group 2: 2.49 MBq/mg
Groups 3: 5.39 MBq/mg
Radiochemical purity: >97%
Source: Selcia
Lot/Batch number: 5210MFO001-1
Structure:



* position of [^{14}C]-label

Vehicle: The oral dose vehicle was 1% carboxymethylcellulose (CMC) containing 0.1% (v/v) Tween 80. The intravenous dose vehicle was dimethylsulfoxide (DMSO): polyethylene glycol (PEG200): saline (10:25:65, (v/v/v)).

Preparation of dosing solutions: Radiolabelled SYN545192 was homogenously suspended in 1% CMC containing 0.1% (v/v) Tween 80 for oral dosing. Radiolabelled SYN545192 was in solution in DMSO : PEG200 : saline (10:25:65, (v/v/v)) for intravenous dosing.

Test Animals:

Species:	Rat
Strain:	Han Wistar
Age/weight at dosing:	8-9 weeks (males); 11-12 weeks (females) Weight range at time of dosing: 257 - 272 g for males; 214 - 236 g for females (1 mg/kg po) 269 - 300 g for males; 204 - 234 g for females (0.25 mg/kg i.v.)
Source:	Charles River (UK) Limited
Housing:	Pre-study: housed singly (Group 2) or in groups of 2/3 (Group 3) in solid bottomed polycarbonate cages with bedding On study: housed singly (Group 2) or in groups of 2 (Group 3) in polycarbonate cages with stainless steel grid bottoms
Acclimatisation period:	6-14 days
Diet:	Rat and Mouse No.1 maintenance diet, Special Diet Services, Stepfield, Witham, Essex, UK. *Ad libitum*.
Water:	Tap water *ad libitum*
Environmental conditions:	Temperature: 20 ± 0°C Humidity: 44 - 69% Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

Experimental dates: Start: 20 September 2011; End: 13 March 2012

Group Arrangements: Animals were assigned to 2 groups as shown in the table below.

Table B.6.1.2./01-1: Dosing groups for pharmacokinetic studies for [^{14}C]-SYN545192

Test Group	Dose (mg/kg)	Number/sex	Remarks
Group 2 Blood and plasma collection, (Oral)	1	4 males, 4 females	Serial blood collections over a time course.
Group 3 Blood and plasma collection (I.V. bolus)	0.25	4 males, 4 females	Serial blood collections over a time course.

Dosing and sample collection: Initially, intravenous administration was by a 1 hour infusion at 0.5 mg/kg (Group 1). The estimates of oral bioavailability following the 0.5 mg/kg i.v. infusion and the 1 mg/kg oral administration were very high (>400%). The concentrations of radioactivity in blood from the 0.5 mg/kg (intravenous infusion) dose group (Group 1) are presented in Appendices 9-10 of the study report. As the oral data generated in this study corresponded with that of previous studies, this suggested that the blood concentrations of radioactivity following intravenous infusion were lower than expected. Therefore, to investigate whether this was an anomalous result or whether it could be confirmed, a bolus intravenous dose was administered at a lower dose of 0.25 mg/kg. A comparison of the blood concentrations following the bolus and infusion intravenous doses showed that they were much lower following the infusion, indicating

the data to be anomalous. Therefore, the intravenous infusion data will not be discussed in the following text.

A single intravenous dose of [^{14}C]-SYN545192 dissolved in DMSO : PEG200 : saline (10:25:65, (v/v/v)) was administered to each rat in Group 3 at a dose rate of 5 mL/kg. A single oral dose of [^{14}C]-SYN545192 suspended in CMC containing Tween 80 was administered to each rat in Group 2 by gavage at a dose rate of 11 mL/kg. Animals in Groups 2 & 3 received a dose corresponding to a nominal dose of 1 and 0.25 mg/kg, respectively. Group 2 animals received a target radioactive dose of 2.5 MBq/kg. Group 3 animals received a target radioactive dose of 1.25 MBq/kg.

Serial blood samples (*ca* 0.1 mL) were removed at time points by venipuncture of a tail vein. Blood was collected into heparinised tubes.

All blood samples were analysed for radioactivity by liquid scintillation counting following sample oxidation.

Pharmacokinetic studies:

As indicated above, data from dose Group 1 (1 hour infusion at 0.5 mg/kg) were not considered in the evaluation of this study as they were considered anomalous. The data on which this conclusion was reached are presented in Appendices 9 & 10 of the study report. The results section therefore focuses on data from dose Groups 2 & 3.

To investigate pharmacokinetics, serial blood samples were collected by tail vein bleeding at the following intervals:

Table B.6.1.2./01-2: Blood collection times for pharmacokinetic studies for [^{14}C]-SYN545192

Dose Route	Nominal dose	Animal numbers		Sampling times (hours after dosing)
		Male	Female	
Oral	1 mg/kg	009-012	013-016	1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72,96
Intravenous	0.25 mg/kg	017-020	021-024	0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96

Table B.6.1.2./01-3: Pharmacokinetic parameters

Parameter	Description of parameter
C_{max}	The maximum observed concentration of total radioactivity measured in whole blood after dosing.
C_0	The theoretical mean total radioactivity at time zero after intravascular bolus dosing only estimated by log-linear back-extrapolation to time zero from the first two observed concentrations in a descending concentration vs time profile, or by assigning the first observed concentration to time 0.
t_{max}	The time after dosing at which the maximum concentration of total radioactivity in whole blood was observed, following oral dosing only.
$AUC_{(0-t)}$	The area under the total radioactivity concentration in whole blood vs time curve from time zero to T_{last} (where T_{last} is the time after dosing at which the last quantifiable concentration of test item was observed estimated by the linear trapezoidal method.)
$AUC_{(0-inf)}$	The area under the total radioactivity in whole blood concentration vs time curve from time zero to infinity: $AUC_{(0-inf)} = AUC_{(0-t)} + (C_{last}/ \text{Lamdaz } (\lambda z))$ where C_{last} = the concentration at time T_{last} and λz = apparent terminal phase rate constant.
AUC extrap (%)	The percentage of AUC estimated by extrapolation of the apparent terminal phase
$t_{1/2}$	The apparent terminal phase half-life.
MRT	Mean residence time estimated from time zero to infinity, calculated following intravenous dosing only.
CL	Clearance: the apparent volume of blood cleared of total radioactivity per unit time following intravenous dosing. Clearance was calculated using $AUC_{(0-inf)}$.
Vss	The apparent volume of distribution of total radioactivity at steady state (for i.v. only)
F	Absolute bioavailability was calculated by: $F\% = [AUC_{(0-inf)oral} \times Dose_{i.v.}]/[AUC_{(0-inf)i.v.} \times Dose_{oral}] \times 100\%$
Rsq	The square of the correlation co-efficient for the apparent terminal phase regression line.

Metabolite characterisation studies: Not applicable.

Statistics: Not applicable.

RESULTS AND DISCUSSION

Pharmacokinetic Studies:

Blood kinetics: The concentration of total radioactivity in whole blood following a single oral or intravenous administration of [^{14}C]-SYN545192 at nominal doses of 1 and 0.25 mg/kg, respectively are presented in the table below.

In males given an oral dose, radioactivity peaked at 4 hours and descended thereafter with a plateau between 6 and 10 hours. In females, radioactivity peaked at 1 hour and descended more or less consistently thereafter. Radioactivity in the blood was consistently lower in females than in males. In animals given an i.v. bolus dose, radioactivity descended slightly more quickly in females than in males.

Table B.6.1.2./01-3: Concentrations of radioactivity in whole blood over a time course after administration of [^{14}C]-SYN545192

	Group mean blood concentrations (µg equivalents of [^{14}C]-SYN545192/g)			
	Group 2: 1 mg/kg, oral		Group 3: 0.25 mg/kg, i.v.	
Sampling time (h)	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
0.083	NA	NA	0.065	0.063
0.25	NA	NA	0.054	0.050
0.5	NA	NA	0.048	0.045
1	0.140	0.130	0.039	0.039
2	0.160	0.123	0.031	0.033
4	0.171	0.110	0.027	0.025
6	0.156	0.105	0.025	0.020
8	0.142	0.098	0.024	0.018
10	0.153	0.094	NA	NA
12	0.134	0.093	0.027	0.017
24	0.088	0.069	0.015	0.013
36	0.073	0.053	0.013	0.011
48	0.065	0.035	0.009	0.009
72	0.042	°0.017[A]	0.007	0.005
96	0.031	0.010	0.005	0.003

°=Mean includes results calculated from data less than 30 dpm above background
A = Includes result of animal 015F, however as <LOD, timepoint was not used in determination of PK
NA = Not Applicable

After oral doses, males had higher C_{max}/C_0 values, higher t_{max} times, longer $t_{1/2}$ times, larger AUC values and higher bioavailability compared to females. Half-lives were relatively consistent in males and females regardless of dose with male half-lives close to twice those of females. The male AUC values were more than 5 times higher in oral dosing then in i.v. doses, while female values were closer to 4 times higher in the oral group than the i.v. group. The extrapolated AUCs were comparable between routes in males and slightly higher in i.v.-dosed females.

Table B.6.1.2/01-4: A comparison of pharmacokinetic parameters in blood following administration of [^{14}C]-SYN545192

	Group 2: 1 mg/kg, oral		Group 3: 0.25 mg/kg, i.v.	
Pharmacokinetic parameter	Male	Female	Male	Female
C_{max}/C_0 (µg equiv/g)	0.172	0.130	0.072	0.073
t_{max} (hours)[A]	3.04	1.00	NA	NA

$t_{1/2}$ (hours)	47.97	25.40	53.57	30.86
$AUC_{(0\text{-}t)}$ (µg equiv.h/g)	6.787	4.393	1.174	1.013
$AUC_{(0\text{-}inf)}$ (µg equiv.h/g)	8.855	4.742	1.529	1.146
AUC Extrapolated (%)	23.26	7.31	22.84	11.19
MRT (h)	NA	NA	64.38	44.02
CL (mL/h/kg)	NA	NA	163.1	198.9
Vss (mL/kg)	NA	NA	10220	8628
F (%)[B]	129 (151)	98.7	NA	NA

A = median value of actual sampling times
B = Bioavailability calculated on average dose normalised AUC for intravenous vs individual oral data ranged from 111-214% in males and 77-125% in females. In males the mean was 151% however, animal 011M (F = 214%) appeared to have a much higher exposure than the other 3 males, therefore when this animal was excluded as an outlier the mean bioavailability in male was 129%
NA = Not Applicable

INVESTIGATORS' CONCLUSION:

The systemic oral bioavailability of total radioactivity, after oral administration of 1 mg/kg [^{14}C] SYN545192, in males of 129% (151% when including outlier 011M) and 99% in females indicates that absorption of total radioactivity was essentially complete.

REVIEWER'S CONCLUSIONS

The reviewer agrees with the investigators' conclusions that the oral bioavailability is essentially complete.

B.6.8.1/05 28 Day Tissue Generation Study in rats

Report:	IIA 5.10.2/01. Robertson B, 2010b. SYN545192: Investigative 28 day dietary study in rats with interim kills. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Laboratory Report No. 30096, 19 August 2010. Unpublished (Syngenta File No. SYN545192_10048). EPA MRID No. 48604588

Guidelines: Non-guideline investigative sub-chronic toxicity study.

GLP: Signed and dated GLP and Quality Assurance statements were provided.

EXECUTIVE SUMMARY

In a subchronic toxicity study SYN545192 (purity 98.3%) was administered in the diet to groups of 25 male and 25 female Han Wistar rats (CrL:WI(Han)) at dose levels of 0, 100, 750 and 1500 ppm.

Mortality and viability were assessed daily and clinical observations, body weights, food and water consumption were assessed regularly throughout the study. Five animals/sex/group were terminated in a random order by exposure to carbon dioxide and severance of major blood vessels on Days 3, 4, 8, 15 and 29 of treatment and underwent a full gross necropsy consisting of a complete external and internal examination which included body orifices (ears, nostrils, mouth, anus, vulva) and cranial, thoracic and abdominal organs and tissues.

Adrenals, brain, epididymes, heart, kidneys, liver, ovaries, spleen, testes, thyroids with parathyroids, thymus and uterus were weighed. A comprehensive range of tissues were taken from all animals and fixed. Additionally, sections of liver, kidney, thyroid and uterus were fixed for 48 hours and processed to paraffin wax block (together with a section of duodenum) for possible future analysis. The remainder of the tissues were snap frozen in liquid nitrogen in RNA-ase free tubes and stored at ca -70°C pending possible future analysis. Blood samples (ca 4 mL) were collected at necropsy via the vena cava for possible future analysis.

There were no mortalities or any treatment-related clinical signs observed during the study.

Body weights were decreased at doses ≥ 750 ppm in males and females. In females, 1500 ppm animals had decreased body weights compared to controls after the first day of treatment and, at 750 ppm, body weights were decreased after the second day of treatment. In males, body weights were decreased compared to controls at doses ≥ 750 ppm from day 17 onwards.

Food consumption was reduced sporadically at 1500 ppm in males. In females, food consumption was decreased at doses ≥ 750 ppm throughout treatment.

Covariant liver weights were increased compared to controls in all dose groups in males and females. Following 2 days of treatment, liver weights were increased at 1500 ppm in males. After 3 days of treatment, liver weights were increased in all male dose groups. After 7 days of treatment, liver weights were increased in all male and female treated dose groups. After 14 days of treatment, liver weights were increased at 1500 ppm in males. After 28 days of treatment, liver weights were increased in all treated groups.

All necropsy findings were typical of spontaneously arising background findings in rats of this age and strain on this kind of study.

This study was conducted to gain supplementary samples for possible future investigative analysis. The report was for informational purposes only and no interpretation of the results has been undertaken.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545192
Description:	Technical, white solid
Lot/Batch number:	TE-6341
Purity:	98.3% a.i
CAS#:	Not reported
Stability of test compound:	Stable until end August 2010 (stored at <10ºC)

Vehicle and/or positive control: The test substance was administered in rat and mouse (modified) No.1 Diet SQC Expanded (Fine Ground).

Test Animals:	
Species	Rat
Strain	Han Wistar (CrL:WI(Han))
Age/weight at dosing	Approximately 7 weeks / 173-235 g (males), 140-191 g (females)
Source	Not reported – Assumption that they are from the Margate colony as was the case in other Charles River Edinburgh studies performed for this chemical
Housing	5 per cage in suspended polycarbonate cages with stainless steel grid tops and solid bottoms containing a separate stainless steel food hopper
Acclimatisation period	13 days
Diet	Rat and mouse (modified) No.1 Diet SQC Expanded (Fine Ground) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 19 – 23ºC Humidity: 32.46 – 70% Air changes: Not reported Photoperiod: 12 hours light/dark cycle

Study Design and Methods:

In-life dates: Start: 18 August 2008 End: 22 September 2008

Animal assignment: Groups of 25 male and 25 female rats were assigned to groups as follows:

Table B.6.8.1/01-1: Study design

Test group	Dietary concentration (ppm)	# male	# female
Control	0 (control)	25	25

Low	100	25	25
Mid	750	25	25
High	1500	25	25

Diet preparation and analysis: SYN545192 was incorporated into blank diet at doses of 0, 100, 750 and 1500 ppm (no further information reported). Samples from diets prepared on day 1 and week 4 were analysed for achieved concentration. Stability of 4 days was assumed at the Sponsor's request. Trial formulations of the lowest and highest concentrations were investigated for stability and homogeneity under a separate protocol.

Concentration analysis results: All concentrations were between -7.2% to +1.0% of nominal concentration.

Homogeneity results: The coefficient of variation was low (4.8% or lower) indicating satisfactory homogeneity.

Stability results: Formulations in the range 100-5000 ppm were stable for 30 days and 10-100 ppm stable for 15 days at ambient temperature in the dark (from Charles River 425169)

Mortality and clinical observations: Viability was recorded twice daily and clinical observations were recorded once weekly from pre-trial until study completion.

Body weight: The body weight of each animal was recorded twice during pre-trial, daily during the first week then twice weekly for the remainder of the treatment period.

Food consumption and test substance intake: Food consumption for each animal was recorded twice during pre-trial, daily during the first week then twice weekly for the remainder of the treatment period.

Water consumption: Water consumption was qualitatively monitored throughout pre-trial and treatment.

Investigations *post mortem:*

Macroscopic examination: Five animals/sex/group were terminated in a random order by exposure to carbon dioxide and severance of major blood vessels on Days 3, 4, 8, 15 and 29 of treatment. All animals underwent a full gross necropsy consisting of a complete external and internal examination which included body orifices (ears, nostrils, mouth, anus, vulva) and cranial, thoracic and abdominal organs and tissues. All gross findings were recorded in descriptive terms, including location(s), size (in mm), shape, colour, consistency and number.

Organ weights: The following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands x2
brain
epididymides x2
ovaries x2
spleen
testes x2

heart
kidneys x2
liver
thymus
thyroids with parathyroids x2
uterus

Paired organs were weighed separately and the sum of the individual organs used for reporting purposes.

Tissue submission: Representative tissues from the following list were taken from all animals and fixed in 10% neutral buffered formalin, unless otherwise stated.

gross lesions
adrenal gland x2
aortic arch
bone marrow smear
brain
cervical lymph node
diaphragm
epididymis x2
eyes x2 (fixed in Davidson's fluid)
Harderian gland (fixed in Davidson's fluid)
heart
duodenum
jejunum
ileum
colon
caecum
rectum
kidney
larynx
liver
lung
lymph node - mesenteric
nasal cavity and pharynx
oesophagus
optic nerve (fixed in Davidson's fluid)
ovary x2
oviducts
pancreas
pituitary gland
prostate gland
rib
sciatic nerve
seminal vesicle
skin and mammary gland
soleus muscle
spinal cord (cervical, midthoracic, lumbar)
spleen
sternum
stomach
testis (fixed in modified Davison's fluid)
thigh muscle
thyroids with parathyroids x2
thymus
tongue
trachea
urinary bladder
uterus
vagina and cervix

All tissues were stored for possible future analysis.

Additionally, sections of liver, kidney, thyroid and uterus were fixed for 48 hours and processed to paraffin wax block (together with a section of duodenum) for possible future analysis. The remainder of the tissues were snap frozen in liquid nitrogen in RNA-ase free tubes and stored at *ca* -70°C pending possible future analysis.

Blood samples (*ca* 4 mL) were collected at necropsy *via* the vena cava, after *ca* 1 hr. at room temperature they were centrifuged and the serum was transferred into 4 tubes. One sample from each animal was sent to Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42

6EY, UK. The remaining samples were frozen and stored at approximately -20°C at Charles River, Edinburgh for possible future analysis.

Statistics: All analyses were two-tailed for significance levels of 5% and 1%.

All means are presented with standard deviations.

Absolute organ weights were analysed initially by a one-way analysis of variance (ANOVA).

Organ weights were also analysed by analysis of covariance (ANCOVA) on final body weight (***Shirley, 1977***). This statistical analysis provided an Adjusted Organ Weight value.

Summary values of body weights, food consumption and organ to body weight ratios are presented but these were not analysed statistically.

For all the parameters evaluated initially by ANOVA or ANCOVA, Dunnett's test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA. The Dunnett's test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant.

The Reviewer agrees with the statistical methods used.

RESULTS AND DISCUSSION

Mortality: There were no mortalities.

Clinical observations: There were no treatment related clinical signs observed during the treatment period. Scabs, lesions and bald areas were seen in all the treatment groups and were consistent with group housing.

Body weight and weight gain: Statistical analyses were not performed for body weight parameters due to the interim kills.

In females at doses ≥ 750 ppm, there was a decrease in body weights compared to controls from day 1 onward at 1500 ppm and day 2 onward at 750 ppm. There was no dose response by the end of the treatment period, but the magnitude of the change was indicative of an adverse and treatment-related change.

In males, body weights were decreased compared to controls at doses ≥ 750 ppm from day 17 onwards.

Table B.6.8.1/01-1: Intergroup comparison of bodyweights (g) - selected timepoints

	Dietary concentration of SYN545192 (ppm)							
	Males				Females			
Day	**0**	**100**	**750**	**1500**	**0**	**100**	**750**	**1500**
0	207 ± 14	206 ± 15	210 ± 16	212 ± 10	166 ± 8	166 ± 9	165 ± 10	162 ± 8
1	211 ± 15	209 ± 15	211 ± 16	206 ± 10	169 ± 8	171 ± 10	163 ± 11	**157 ± 8 (↓7)**
2	217 ± 15	216 ± 15	216 ± 15	209 ± 10	172 ± 9	176 ± 10	**163 ± 10 (↓5)**	**154 ± 8 (↓10)**
7	240 ± 16	243 ± 16	237 ± 19	228 ± 12	183 ± 13	187 ± 9	**169 ± 10 (↓8)**	**164 ± 7 (↓10)**
14	260 ± 19	267 ± 20	256 ± 22	251 ± 12	198 ± 13	201 ± 11	**181 ± 9 (↓9)**	**179 ± 8 (↓10)**
17	273 ± 26	274 ± 32	**257 ± 24 (↓6)**	**257 ± 17 (↓6**	210 ± 11	204 ± 15	**180 ± 7 (↓14)**	**180 ± 9 (↓14)**
28	299 ± 31	306 ± 37	**281 ± 26 (↓6)**	**276 ± 20 (↓8)**	226 ± 14	217 ± 16	**186 ± 7 (↓18)**	**187 ± 11 (↓17)**

[a] Data obtained from pages 19 – 22 of the study report

Food consumption and compound intake: Statistical analyses were not performed for food consumption parameters due to the interim kills.

Food consumption was decreased in the first 5 days in 1500 ppm males. In females, food consumption was decreased from day 1 until the end of the treatment period at doses ≥ 750 ppm.

Table B.6.8.1/01-2: Intergroup comparison of food consumption (g/animal/day) – selected timepoints

	Dietary concentration of SYN545192 (ppm)							
	Males				Females			
Day	**0**	**100**	**750**	**1500**	**0**	**100**	**750**	**1500**
0	23.0 ± 3.4	22.1 ± 0.9	23.1 ± 4.1	26.0 ± 6.3	18.5 ± 1.8	19.1 ± 1.3	20.3 ± 2.4	19.0 ± 2.1
1	21.0 ± 2.3	21.3 ± 0.8	14.8 ± 2.1	**9.1 ± 3.2 (↓57)**	15.7 ± 2.0	15.4 ± 1.1	**8.5 ± 4.1 (↓46)**	**5.7 ± 2.7 (↓64)**
5	22.3 ± 4.2	20.5 ± 3.8	20.9 ± 1.0	**17.9 ± 2.5 (↓20)**	17.0 ± 0.9	16.7 ± 1.1	**14.5 ± 2.9 (↓20)**	**17.7 ± 6.5 (↓48)**
7	18.4 ± 10.4	24.3 ± 2.7	24.6 ± 1.4	23.8 ± 5.2	18.1 ± 2.1	19.5 ± 1.9	**14.8 ± 3.2 (↓18)**	**15.5 ±5 .6 (↓14)**
14	21.3 ± 0.8	22.9 ± 1.8	22.6 ± 0.4	21.5 ± 5.4	21.2 ± 4.0	19.5 ± 2.3	20.3 ± 1.6 (↓4)	**14.6 ± 1.7 (↓31)**
28	21.0[b]	21.9	19.5	19.5	19.5	16.8	**15.5 (↓21)**	**12.7 (↓35)**

[a] Data obtained from pages 23 – 26 of the study report
[b] No statistical significance as food consumption was the average of two animals in one cage

Water consumption: Water consumption was monitored visually and not quantitatively. According to the study authors, there was no observable difference in water consumption between the groups.

Sacrifice and pathology:

Organ weights: There were sporadic statistically significant and/or dose-reponsive changes in organ weights in the adrenals, kidneys, thymus and thyroids; however, the changes were sporadic, lacked dose-response and/or lacked a time-response.

The only consistent change was in the covariant analysis of liver weights in males and females. Liver weights were increased in all dose groups. In males, high-dose animals had increased liver weights as of day 3 and all other dose groups were increased as of day 4, with the exception of day 15. In females, liver weights were increased in all dose groups at day 8 and 29.

Table B.6.8.1/01-3: Intergroup comparison of selected organ weights (covariant analyses; g)

Organ		Dietary concentration of SYN545192 (ppm)							
		Males				Females			
	Day	0	100	750	1500	0	100	750	1500
Heart	3	0.78 ± 0.01	0.77 ± 0.01	0.74 ± 0.01	0.74 ± 0.01	0.61 ± 0.02	0.63 ± 0.02	0.59 ± 0.02	0.57 ± 0.02
	4	0.71 ± 0.03	0.75 ± 0.03	0.71 ± 0.03	0.72 ± 0.03	0.63 ± 0.02	0.63 ± 0.02	0.60 ± 0.02	0.59 ± 0.03
	8	0.77 ± 0.08	0.81 ± 0.07	0.91 ± 0.07	0.81 ± 0.08	0.65 ± 0.02	0.65 ± 0.02	0.61 ± 0.02	0.61 ± 0.02
	15	0.83 ± 0.02	0.84 ± 0.02	0.81 ± 0.02	0.84 ± 0.02	0.68 ± 0.02	0.68 ± 0.03	0.65 ± 0.02	0.67 ± 0.02
	29	0.73 ± 0.06	0.88 ± 0.07	0.89 ± 0.07	0.88 ± 0.07	0.70 ± 0.03	0.75 ± 0.02	0.67 ± 0.03	0.68 ± 0.03
Kidneys	3	1.82 ± 0.04	1.73 ± 0.05	1.69 ± 0.04	1.68 ± 0.05	1.38 ± 0.05	1.36 ± 0.07	1.40 ± 0.05	1.37 ± 0.08
	4	1.61 ± 0.06	1.73 ± 0.06	1.65 ± 0.06	1.60 ± 0.06	1.34 ± 0.05	1.40 ± 0.05	1.40 ± 0.05	1.45 ± 0.07
	8	1.71 ± 0.03	1.77 ± 0.03	1.70 ± 0.03	1.74 ± 0.03	1.35 ± 0.04	1.27 ± 0.04	1.40 ± 0.04	1.44 ± 0.04
	15	1.91 ± 0.04	1.77 ± 0.04	1.87 ± 0.04	1.86 ± 0.04	1.49 ± 0.04	1.50 ± 0.04	1.33 ± 0.4*	1.27 ± 0.04**
	29	1.95 ± 0.05	1.85 ± 0.05	2.00 ± 0.05	1.93 ± 0.05	1.37 ± 0.06	1.40 ± 0.05	1.45 ± 0.06	1.39 ± 0.06
Liver	3	9.32 ± 0.40	10.40 ± 0.42 (↑12)	10.02 ± 0.40 (↑8)	**10.99 ± 0.41* (↑18)**	6.42 ± 0.21	7.53 ± 0.26** (↑17)	6.88 ± 0.21 (↑7)	6.79 ± 0.30 (↑6)
	4	8.81 ± 0.38	**9.98 ± 0.38 (↑13)**	**10.28 ± 0.39* (↑17)**	**10.34 ± 0.38* (↑17)**	6.99 ± 0.19	7.23 ± 0.23 (↑3)	7.60 ± 0.19 (↑9)	7.59 ± 0.29 (↑9)
	8	9.77 ± 0.29	**10.98 ± 0.28* (↑12)**	**10.82 ± 0.28* (↑11)**	**12.34 ± 0.30** (↑26)**	6.89 ± 0.24	**7.78 ± 0.26* (↑13)**	**7.85 ± 0.22* (↑14)**	**7.98 ± 0.26* (↑16)**
	15	10.39 ± 0.45	10.24 ± 0.47 (↓1)	12.03 ± 0.44 (↑16)	**13.56 ± 0.46** (↑31)**	7.97 ± 0.26	7.87 ± 0.32 (↓1)	8.41 ± 0.27 (↑6)	8.61 ± 0.30 (↑8)
	29	10.86 ± 0.51	**12.37 ± 0.53 (↑14)**	**12.03 ± 0.51 (↑11)**	**13.85 ± 0.52** (↑28)**	7.28 ± 0.31	**8.31 ± 0.27* (↑14)**	**8.70 ± 0.29* (↑20)**	**9.28 ± 0.29** (↑27)**
Thymus	3	0.626 ±	0.564 ±	0.482 ±	0.632 ±	0.486 ±	0.536 ±	0.459 ±	0.481 ±

		0.042	0.043	0.042	0.043	0.030	0.038	0.030	0.043
	4	0.581 ± 0.020	0.578 ± 0.020	0.523 ± 0.020	0.505 ± 0.20*	0.480 ± 0.027	0.453 ± 0.032	0.493 ± 0.027	0.512 ± 0.040
	8	0.557 ± 0.039	0.554 ± 0.038	0.574 ± 0.038	0.531 ± 0.041	0.502 ± 0.045	0.509 ± 0.044	0.513 ± 0.042	0.482 ± 0.050
	15	0.614 ± 0.038	0.551 ± 0.041	0.482 ± 0.038	0.512 ± 0.039	0.529 ± 0.046	0.471 ± 0.056	0.563 ± 0.048	0.537 ± 0.053
	29	0.439 ± 0.032	0.475 ± 0.033	0.466 ± 0.032	0.470 ± 0.033	0.462 ± 0.038	0.403 ± 0.033	0.369 ± 0.035	0.436 ± 0.035
Thyroid	3	0.0146 ± 0.0021	0.0182 ± 0.0025	0.0174 ± 0.0021	0.0207 ± 0.0022	0.0115 ± 0.0032	0.0125 ± 0.0036	0.0136 ± 0.0029	0.0203 ± 0.0041
	4	0.0162 ± 0.0022	0.0163 ± 0.0022	0.0147 ± 0.0023	0.0153 ± 0.0025	0.0123 ± 0.0016	0.0140 ± 0.0020	0.0153 ± 0.0018	0.0136 ± 0.0024
	8	0.0155 ± 0.0020	0.0188 ± 0.0020	0.0187 ± 0.0020	0.0170 ± 0.0021	0.0107 ± 0.0031	0.0124 ± 0.0026	0.0132 ± 0.0024	0.0191 ± 0.0030
	15	0.0169 ± 0.0022	0.0180 ± 0.0027	0.0221 ± 0.0021	0.0220 ± 0.0023	0.0181 ± 0.0017	0.0153 ± 0.0020	0.0143 ± 0.0019	0.0178 ± 0.0019
	29	0.0178 ± 0.0027	0.0226 ± 0.0028	0.0173 ± 0.0027	0.0221 ± 0.0028	0.0159 ± 0.0034	0.0137 ± 0.0029	0.0199 ± 0.0031	0.0165 ± 0.0031

* Statistically significant difference from control group mean, $p<0.05$

** Statistically significant difference from control group mean, $p<0.01$

Bolded values determined to be treatment-related

Macroscopic findings: A small number of lesions were observed, none of which was related to treatment.

INVESTIGATORS' CONCLUSION: This study was conducted to gain supplementary samples for possible future investigative analysis. The report was for informational purposes only and no interpretation of the results has been undertaken.

REVIEWER'S CONCLUSION: In conclusion, changes following up to 28 days of treatment with SYN545192 were limited to body weight, food consumption and liver weights in the parameters observed in this study. Body weights were decreased at doses ≥ 750 ppm in males and females, food consumption was decreased at doses ≥ 750 ppm in females and 1500 ppm in males, and liver weights were increased in all doses in males and females.

Reference:

Shirley E (1977) The Analysis of Organ Weight Data. Toxicology 8 p13-22.

CSAA798670 (Metabolite of SYN524464)

B.6. Toxicology and metabolism

B.6.3 Short-time toxicity

28 Day Oral (Dietary) Toxicity Study in Wistar Rat

Short-term toxicity

ORAL 28-DAY TOXICITY (RODENTS)

28 DAY ORAL TOXICITY

Study Type: CSAA798670 - Repeated Dose 28-Day Oral Toxicity Study in rodents. EPA OPPTS 870.3050 (2000); OECD 407 (1995).

Test Material: Content of **CSAA798670** 97.4%

Synonyms: **CSAA798670** (Metabolite of SYN545192)

Citation: CSAA798670 – 28 Day Oral (Dietary) Toxicity Study in Wistar Rat. Harlan Laboratories Ltd. Zelgliweg 1. 4452 Intigen, Switzerland. Laboratory report number: C57852 Study Completion Date: January 11, 2011.

Sponsor: Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

Report Number: C57852

Executive Summary – A 28 Day Oral (Dietary) Toxicity Study was conducted with WIST (SPF) rats to determine the acute oral toxicity of CSAA798670. Rats were treated with a daily (dietary) dose of CSAA798670 at the dose levels of 2000, 6000 and 12000 ppm, for 28 days.
Observations for viability/ mortality were recorded twice daily. The animals were observed for clinical signs once daily during both, acclimatization and the treatment period. Forelimb and hind limb grip strength, locomotor activity, food consumption, body weight and body weight were determined.
Animals were examined during the acclimatization period and during the final week of the study for ophthalmic abnormalities. A complete blood cell count, coagulation, clinical biochemistry parameters, and urine parameters were also determined. All animals were weighed and necropsied. Necropsy included examination of the external surface of the body, all orifices and the cranial, thoracic and abdominal cavities and their contents.
Organs were weighted, and tissue samples were examined, processed, embedded and cut at an approximate thickness of 2 - 4 micrometers and stained with hematoxylin and eosin.

No deaths occurred during the course of the study, and there were no treatment related effects on hematology, clinical chemistry or organ weights.
Hepatocellular hypertrophy was noted in livers of males and females at 6000 and 12000 ppm, accompanied by a lower incidence of glycogen deposition at 12000 ppm. However, considering the absence of any effect on liver weight or clinical chemistry parameters related to liver function, minimal hypertrophy does not represent an adverse effect.

Based on the results, the no observed adverse effect level (NOAEL) associated with dietary CSAA798670 exposure in rats is 12000 ppm, corresponding to 1007 mg/kg/day in males and 1043 mg/kg/day in females.

Compliance: Signed and dated Good Laboratory Compliance, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

Materials and Methods

Materials

Test material:	CSAA798670 (CA4312D)	
Physical Description:	Light brown powder	
Lot/Batch #:	LT-DFPA09001	
Purity:	97.4%	
Test species:	Rat	
Strain:	RccHan: WIST(SPF)	
Sex:	21 males, 21 females	
Age at dosing:	7 weeks	
Weight at dosing:	Males: 195.5 – 227.5 g Females: 143.7 – 175.9 g	
Source:	Harlan Laboratories, Inc.	
Housing:	In groups of five in Makrolon type-4 cages with wire mesh tops and standard softwood bedding	
Diet:	Powdered standard Kliba Nafag 3433 rodent maintenance diet, batch no. 42/09 was available *ad libitum*.	
Water:	Community tap water from Itingen was available *ad libitum* in water bottles.	
Environmental conditions:	Temperature: Humidity: Ventilation: Photoperiod:	22 ± 3 ºC 30-70% 10-15 air exchanges/hour 12-hour fluorescent light/dark cycle with music during the light period
Acclimation:	Under test conditions after health examination. Duration: 7 days	

Study design and methods

Study experimentation dates - Start: August 26, 2009 End: January 11, 2010

Treatment

Dose Calculations

The dose levels were selected based on a previous dose range finding toxicity study with CSAA798670 in Wistar rats, Harlan Laboratories study C46185, using dose levels of 4000, 8000 and 16000 ppm, resulting in a NOEL of 16000 ppm.

Analysis of dose concentration and homogeneity was carried out on all dose groups. The samples were analyzed using a method determined at Harlan Laboratories Ltd., Itingen / Switzerland.

The achieved concentration of CSAA798670 in diet batches was found to range from 89.0% to 109.8% of the nominal concentration. Two batches were only marginally outside the acceptable range of ±10% of the nominal concentration (-10.2% and -11% for the 6000 and 12000 ppm diet batches prepared for the fourth week of dosing, respectively). CSAA798670 was found to be homogeneously distributed in the diet preparations as single sample results did not deviate more than 10% from the corresponding mean, with the exception of the 2000 ppm diet batch prepared for the fourth week of dosing in which one out of three samples deviated marginally from the corresponding mean by 14.1%. This minor deviation did not adversely affect the outcome of the study.

Dosing

The animals received a daily dose of the test item. Doses were prepared as follows:

- 2000 ppm (243.59 g of blank diet with 6.41 g of CSAA798670. Mixed with 2.956 kg of blank diet)
- 6000 ppm: (230.68 g of blank diet and 19.32 g of CSAA798670. Mixed with 2.95 kg of blank diet)
- 12000 ppm (211.1 g of blank diet and 38.9 g of CSAA798670. Mixed with 2.989 kg of blank diet)

Group 1 (5 males and 5 females): 0 ppm
Group 2 (5 males and 5 females): 2000 ppm
Group 3 (5 males and 5 females): 6000 ppm
Group 4 (5 males and 5 females): 12000 ppm

Allocation and dose levels

Allocation and dose levels	**Group 1 (control)**	**Group 2**	**Group 3**	**Group 4**
ppm	**0**	**2000**	**6000**	**12000**
Males	1-5	6-10	11-15	16-21
Females	21-25	26-30	31-35	36-40

The following overall (week 1-4) mean values of doses received over treatment were achieved:

Group	**Dietary concentration**	**Males**	**Females**
Nos.	**(ppm)**	**Nominal test item intake (mg/kg/day)**	**Nominal test item intake (mg/kg/day)**
1	0		
2	2000	167	175
3	6000	511	572
4	12000	1007	1043

Statistical Analysis – The statistical analyses were performed with SAS version 9.1.3. All procedures were done with proc glm (for diagnostic homogeneity tests of the variance according to Bartlett) and with proc mixed (since by this approach the Dunnett's tests can be directly assessed). All analyses were two-tailed for significance levels of 5% and 1%. Body weights, cumulative body weight gain, food utilization, quantitative FOB measurements (grip strength), motor activity data at each measurement interval and overall activity, and absolute organ weights were analyzed initially by a one-way analysis of variance (ANOVA). Organ weights were also analyzed by analysis of covariance (ANCOVA) on final body weight. For all of the parameters evaluated initially by ANOVA or ANCOVA, the Dunnett's test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA.

Clinical pathology

Blood and urine samples were obtained prior to terminal kill, under light isoflurane anesthesia. The animals were fasted in metabolism cages for approximately 18 hours before blood sampling but allowed access to water *ad libitum*.

The following parameters were determined:

hematology parameters	biochemistry parameters
Erythrocyte count	Glucose
Hemoglobin	Urea
Hematocrit	Creatinine
Mean corpuscular volume	Bilirubin, total
Mean corpuscular hemoglobin	Cholesterol, total
Mean corpuscular hemoglobin concentration	Triglycerides
Reticulocyte count	Aspartate aminotransferase
Erythrocyte morphology	Alanine aminotransferase
Hemoglobin distribution width	Alkaline phosphatase
Differential leukocyte count:	Sodium
Neutrophils	Potassium
Eosinophils	Chloride
Basophils	Calcium
Lymphocytes	Phosphorus
Monocytes	Protein, total
Large unstained cells	Albumin
Platelet count	Globulin
Leukocyte count, total	Albumin/Globulin ratio

Samples of the following tissues and organs were collected from all animals at necropsy:

Tissues/organs	Weighted	Collected	Examined
Adrenal glands	√	√	√
Bone (sternum, femur including joint)		√	√
Bone marrow (sternum, femur)		√	√
Brain - including section of medulla/pons, cerebral and cerebellar cortex	√	√	√
Cecum	√	√	√
Colon		√	√
Duodenum		√	√
Epididymides(fixed in Bouin's solution)	√	√	√
Eye (fixed in Davidson’s solution)		√	√
Heart		√	√
Ileum, with Peyer’s patches		√	√
Jejunum with Peyer’s patches		√	√
Kidneys	√	√	√
Liver		√	√
Lungs, filled w/formalin at necropsy		√	√
Lymph nodes – mesenteric and mandibular	√	√	√
Ovaries w/oviducts	√	√	√

Prostate + seminal vesicles w/coagulating glands		√	√
Rectum		√	√
Sciatic nerve		√	√
Spinal cord- cervical, midthoracic, lumbar	√	√	√
Spleen		√	√
Skeletal muscle		√	√
Stomach		√	√
Testes (fixed in Bouin's solution)		√	√
Thymus		√	√
Thyroid (incl. parathyroid gland, if possible)		√	√
Trachea		√	√
Urinary bladder, filled w/formalin at necropsy		√	√
Uterus, including cervix		√	√
Vagina		√	√
All gross lesions and masses		√	√

Results and Discussion

Mortality

All animals survived to the exposure.

Clinical observations

2000 ppm

- Clinical signs (daily): No treatment-related clinical signs were observed
- Detailed clinical observations (weekly): No treatment-related clinical observations were found
- Functional observation battery (week 4): No treatment-related clinical observations were found

6000 ppm

- Clinical signs (daily): No treatment-related clinical signs were observed
- Detailed clinical observations (weekly): Miosis in both eyes of two females was noted during week 3 and 1 to 3 respectively
- Functional observation battery (week 4): Miosis was noted in both eyes of one female.

12000 ppm

- Clinical signs (daily): No treatment-related clinical signs were observed
- Detailed clinical observations (weekly): Miosis was noted during week 2 and 3 in both eyes of one male and two females during weeks 2 to 3 and weeks 1 to 3 respectively.
- Functional observation battery (week 4): Miosis was noted in both eyes of two males

No treatment-related effects were present in Grip strength in the fore- and hind limbs, locomotor activity and food consumption

Hematology/ Biochemistry parameters

No treatment-related effects were present in all hematology and biochemistry parameters investigated. Slight differences from control values in some hematology parameters were noted in the intermediate dose groups. There were not treatment-related effects, because the values were within the reference range.

Body and organ weight

Body weight and organ weights, were not affected by CSAA798670 consumption at any dose.

Organ weights and body weight (grams) in MALES:

Tissues/organs	Group 1 0 ppm	Group 2 2000 ppm	Group 3 6000 ppm	Group 4 12000 ppm
Body weight	316.4	333.3	319.0	321.6
Brain	0.62	0.60	0.62	0.63
Heart	0.31	0.31	0.32	0.32
Liver	3.05	3.18	3.34	2.70
Thymus	0.150	0.167	0.167	0.166
Kidneys	0.62	0.54	0.65	0.63
Adrenals	0.021	0.020	0.020	0.020
Spleen	0.22	0.25	0.24	0.21
Testes	1.12	1.08	1.02	1.06
Epididymis	0.320	0.333	0.329	0.320

Organ weights and body weight (grams) in FEMALES:

Tissues/organs	Group 1 0 ppm	Group 2 2000 ppm	Group 3 6000 ppm	Group 4 12000 ppm
Body weight	202.7	205.5	199.9	206.0
Brain	1.86	1.83	1.80	1.85
Heart	0.71	0.77	0.68	0.76
Liver	6.59	7.33	6.62	7.01
Thymus	0.394	0.412	0.352	0.443
Kidneys	1.31	1.38	1.34	1.35
Adrenals	0.074	0.071	0.081	0.073
Spleen	0.51	0.44	0.49	0.46
Ovaries	0.103	0.102	0.100	0.104
Uterus	0.72	0.87	0.75	0.82

Macroscopical and microscopical findings

0 ppm (control group)

- Macroscopical findings: The liver in one male was enlarged. Furthermore, an isolated and reddish focus with a diameter of 1 mm was observed in the thymus of one female of the control group.
- Microscopic findings: Lymphocytolysis in the thymus occurred in one female. Minimal tubular degeneration was noted in the testes in one male.

2000 ppm

- Macroscopical findings: Findings were restricted to an enlarged liver in two males and several dark red foci with a size of 1x2 mm were recorded in the stomach of one male. These findings are not

considered to be treatment-related changes as there was no clear dose response-relationship present and the alterations were of minor incidence.
- Microscopic findings: No treatment-related clinical observations were found at this dose

6000 ppm

- Macroscopical findings: Minor findings were restricted to an enlarged liver in one male and an isolated and dark red focus with a diameter of 1 mm was observed in the thymus of one male. These findings are not considered to be treatment-related changes as there was no clear dose response-relationship present and the alterations were of minor incidence
- Microscopic findings: In the liver, centrilobular hepatocellular hypertrophy was noted in 2 males and one female. There were no further microscopic findings in rats that could be correlated to treatment with CSAA798670.

12000 ppm

- Macroscopical findings: Several dark red foci with a diameter of 1 mm were present in the thymus of one male. This finding is not considered to be treatment-related change as there was no clear dose response-relationship present.
- Microscopic findings: Centrilobular hepatocellular hypertrophy was noted in 3 females at 12000 ppm. One female had diffuse hepatocellular hypertrophy accompanied by hepatocellular pigment. The hypertrophy was associated with a decrease in the incidence of hepatocellular glycogen deposition. Additionally, ingible body macrophages (minimal grade) in the mesenteric lymph node were noted in one male and one female. Lymphocytolysis in the thymus occurred in two females. Minimal tubular degeneration was noted in the testes in two males. There were no further microscopic findings in rats that could be correlated to treatment with CSAA798670.

Investigator's Conclusions – Based on the results of this study, the no observed adverse effect level (NOAEL) associated with dietary CSAA798670 exposure in rats is 12000 ppm, corresponding to 1007 mg/kg/day in males and 1043 mg/kg/day in females.

Reviewer's Conclusions – This 28 Day Oral (Dietary) Toxicity Study is classified as acceptable. This study satisfies the guideline requirement for a 28 Day Oral (Dietary) Toxicity Study (EPA OPPTS 870.3050; OECD 407) in the rat.

Deficiencies - None

STUDY TYPE: Rodent and Human *in vitro* Dermal Absorption Studies

TEST MATERIAL: Benzovindiflupyr (BZV)

SYNONYMS: Solatenol

CITATION: I.R. Johnson, Feb. 7, 2012. SYN545192 EC (A17056F) – *In Vitro* Absorption through Rat Epidermis Using [^{14}C]-Radiolabelled SYN545192. Final Report. Feb. 7, 2012. Report Number JV2150-REG. Unpublished.

I.R. Johnson, Feb. 7, 2012. SYN545192 EC (A17056F) – *In Vitro* Absorption through Human Epidermis Using [^{14}C]-Radiolabelled SYN545192. Final Report. Feb. 7, 2012. Report Number JV2148-REG. Unpublished.

SPONSOR: Syngenta Crop Protection, LLC, 410 Swing Road, Greensboro, NC

EXECUTIVE SUMMARY:

The objective of this study was to determine the *in vitro* absorption of SYN454192 (Benzovindiflupyr) through human and rat skin over a period of 24 hours. Dorsal skin samples from male Wistar rats and thigh or abdominal skin samples from human donors were treated with 10 µL/cm^2 skin of ^{14}C-SYN545192 at one of three doses (0.001, 0.01 and 1.5 mg ai/cm^2). Static glass diffusion cell systems with an exposed membrane area of 2.54 cm^2 and a volume of approximately 4.5 mL were used in this study.

Mean recoveries for each group (dose level and species) ranged from 90.8 to 103%. All means fell within the acceptable range of 100 +/- 10% as determined by the OECD Guideline 428. Therefore, study results were not adjusted for incomplete recovery.

Results of this study suggest that SYN545192 is readily absorbed through rat skin, and that dermal absorption is higher in rats than in humans. The pattern of absorption also appears to be similar between rats and humans, with saturation at higher doses (i.e. greater dermal absorption at lower doses).

The mean dermal absorption in the rat skin test system was 12.0% for the high dose, 28.6% for the medium dose and 56.5% for the low dose. The majority of the applied dose was found in the skin wash for the medium and high doses (70% and 84%, respectively), but for the low dose the majority was found in the receptor fluid (48%).

The mean total absorbed dose for human skin was 0.55% for the high dose, 0.63% for the medium dose and 3.17% for the low dose. The majority of the administered dose was recovered from the skin wash (96 to 100%) for all dose levels.

The study report was lacking in several details, but no major study limitations were identified. It is recognized that *in vitro* dermal absorption studies, alone, are not sufficiently validated for use in deriving estimates of systemic exposure for risk assessments (NAFTA, 1999). As such, the results of this study alone are of limited use. *In vitro* studies can be used by PMRA in a 'triple-pack' approach with an *in vivo* study (NAFTA, 2008).

EPA Conclusion: Correcting for tapes 1 & 2, the mean total absorbed dose for human skin was 0.47% for the high dose, 0.56% for the medium dose and 2.77% for the low dose.

GUIDELINE OR PROTOCOL FOLLOWED:

1) OECD Guideline 428 (2004): Skin absorption: *In vitro* method
2) EPA (August, 1998) Health Effects Test Guidelines, OPPTS 870-7600 Dermal Penetration
3) European Commission Guidance Document on Dermal Absorption (2004) Sanco/222/2000 rev. 7 (19 March, 2004)
4) OECD Document number 28 (2004b): The conduct of skin absorption studies; Organisation for Economic Cooperation and Development, Paris (ENV/JM/MONO(2004)2.

MATERIALS AND METHODS

MATERIALS

Test Material:

Radiolabelled Benzovindiflupyr

Description: [pyrazole-5-^{14}C]-SYN545192
Batch #: 5173GAR001-1
Purity: 96.8% at molecular weight 400.11
Storage: Deep frozen (<-15˚C)
Vehicle/Solvent used: Blank 150 EC formulation which contained all ingredients with the exception of the active ingredient.
Radiolabelling: Distributed in the pyrazole ring
Specific Activity: 19.44 MBq/mL (5.39 mBq/mg)
Radiochemical Purity: 99.7% Confirmed by HPLC
Source: Selicia Limited, Ongar, Essex, UK.

Non-Radiolabelled Benzovindiflupyr

Description: SYN545192
Lot #: Batch No. SMU9BP005
Purity: 97.0%
Storage: Room temperature
Molecular Name : 3-difluoromethyl-1methyl-1H-pyrazole-4-carboxylic acid (9-dichloromethen-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide
Chemical structure: $C_{18}H_{15}CL_2F_2N_3O$
Vehicle/Solvent used: Blank 150 EC formulation which contained all ingredients with the exception of the active ingredient.
Expiry Date: February 28, 2013.
Manufacturer: Not specified
Source: Sponsor (Syngenta Crop Protection, Switzerland)

Blank Formulation:

Description: SYN545192 EC. Breakdown of components not provided, but it was stated that the blank formulation containing all the ingredients of the 150 EC commercial formulation with the exception of the active ingredient.
Lot/Batch #: TS00023/003
Stability: Not reported.
Source: Sponsor (Syngenta Crop Protection, Switzerland)
Other comments: Stored at room temperature

Relevance of Test Material to Proposed Formulation(s):

The formulation used in this study is an emulsifiable concentrate containing 150 g/L of the active ingredient SYN545192 (Benzovindiflupyr). Details were not presented on the rest of the ingredients in the formulation.

There are several formulations containing benzovindiflupyr proposed for use in Canada. They are formulated as emulsifiable concentrates, soluble liquid and wettable granules. Products contain less than 150 g/L of the active ingredient and 6 of the 8 products are coformulations with difenoconazole, azoxystrobin, or propiconazole.

Test Samples:

Rat Skin
Species: Rat
Strain: Wistar Crl:(WI)
Gender: Male
Age and weight at study initiation: 28 days; weight not reported
Source: Charles River UK Ltd, Margate, Kent, UK
Environmental conditions: Not Stated

Human Skin
Species: Human
Gender and Age: Female (69-83 years)
Source: Whole skin samples (thigh and abdomen) obtained from a tissue bank

STUDY DESIGN

No deviations from the study protocols were provided in the study report.

Dose

Rationale: Dose selection was based on the formulation concentrate and the 1/150 and 1/1500 aqueous dilutions of the formulation concentrate.

Nominal Doses: 0.001, 0.01, and 1.5 mg ai/cm^2 skin.

Actual Doses: Actual dose concentrations were determined by LSC. However, the study does not report weighing the pipette tip used for application, before and after dosing. Therefore, the actual doses delivered to the membranes cannot be determined. Assuming the entire dose was delivered, the actual doses are 0.000986, 0.0101, and 1.537 mg ai/cm^2 skin (see Table 6.12.2-1 for details).

Dose volume: 25.4 µL/2.54 cm^2 skin (10 µL/cm^2 skin)

Duration of exposures (time from dose to skin wash): 24 hours

Number of donors/group: 3 rats per group
3-4 donors per group
Number of tissue samples/group: For rats: 6 samples from at least 3 different animals per application group for a total of 18 samples
For human samples: 6 samples from at least 3 different donors

Skin Sample Preparation

Clipped, full thickness, dorsal skin samples from male rats aged 28 days was supplied by Charles

River UK Ltd. The skins were soaked for approximately 20 hours in 1.5 M sodium bromide then rinsed in distilled water. The epidermis was carefully peeled from the dermis. Each membrane was given an identifying number and stored frozen at approximately– 20°C on aluminum foil until required for use.

Human skin samples were received from a tissue bank. Skin samples were from female donors aged 69-83 years of age. Samples had been in storage for 1-3 years. Samples were immersed in water at 60 °C for 40-45 seconds and the epidermis teased away from the dermis. Each membrane was given an identifying number and stored frozen, at approximately – 20°C on aluminum foil until required for use.

Membrane integrity was determined by measurement of the electrical resistance across the skin membrane. Membranes with a measured resistance of < 2.5 kΩ for rat skin and <10 kΩ for human skin were regarded as having a lower integrity than normal and not used for exposure to the test materials, due to the possibility of compromised barrier function.

Dose Preparation, Administration and Quantification

Preparation: The high dose solution was prepared by mixing the stock solution of radiolabelled with non-labelled SYN545192 and the blank formulation. The medium dose solution was prepared by mixing the stock solution of radiolabelled SYN545192 with the blank formulation and water (resulting in a dilution ration of 1:150 w/v). The low dose solution was prepared by mixing the stock solution of radiolabelled SYN545192 with the blank formulation and water (resulting in a dilution ratio of 1:1500 w/v). The preparations were mixed on a whirlimixer for 5-10 minutes, glass beads were added to aid mixing.

Triplicate aliquots were added to 10 mL Goldstar scintillation fluid and analysed for radioactivity content by LSC. The results were used to confirm that homogeneity was acceptable.

The time between dose preparation and application was no longer than 7 days. The pH of the formulated doses was not discussed.

Application: 25.4 µL aliquots of the formulated doses were applied manually to each skin membrane using a positive displacement pipette. The dose was applied crop-wise over the skin to maximise coverage. It is unknown if the pipette was used to spread the test material and if it was weighed before and after treatment to determine the actual dose administered (weights were not reported). The donor chambers were left unoccluded.

Quantification: Radiochemical purity, homogeneity, volatility and radioactivity of the ^{14}C-SYN545192 formulated doses were measured by analysing mock doses prepared prior to the main phase of the study by HPLC. Radioactive chemical purity was confirmed to be 99.78%, 99.76% and 99.46% for the concentrate, the 1/150 v/v dose and the 1/1500 v/v dose, respectively. Reanalysis after 24 hours confirmed that the preparations were stable. No significant proportion of the applied SYN545192 was lost due to volatility over 24 hours. Homogeneity of the radioactivity in the dose preparations was assessed and in all cased was regarded as acceptable.

The radioactivity (dpm) and homogeneity of the application doses were determined by LSC before and after treatments. LSC analysis of the mock dose preparations confirmed that the activity was as expected (within 10% of calculated values).

Diffusion Cell Apparatus

Static glass diffusion cell systems with an exposed membrane area of 2.54 cm^2 and a volume of approximately 4.5 mL were used in this study. Discs of approximately 3.3 cm diameter of prepared skin samples were mounted, dermal side down. Diffusion cells were held together with individually numbered clamps and placed in a water bath maintained at 32 ˚C ± 1 ˚C.

The receptor chambers of the cells containing small magnetic stirrer bars were filled with a recorded volume of receptor fluid (50% ethanol in water) and placed in a water bath maintained at a temperature of 32 ˚C ± 1 ˚C. A pre-treatment sample was taken from each receptor chamber for analysis by LSC. The volume reduction in the receptor chamber was immediately compensated by fresh receptor fluid.

Since SYN545192 is poorly soluble in water, the selected receptor fluid was 50% ethanol in water. The solubility of SYN545192 in the receptor fluid was deemed to be acceptable as 1 mg of SYN545192 dissolved in 4.5 mL 50% ethanol in water. The applicant states that the solubility limit is generally regarded as at least 20% of the applied dose being able to dissolve in a volume of receptor fluid (nominally 4.5 mL). A maximum of approximately 4 mg SYN545192 would have been applied to the skin surface from the concentrate formulation.

Skin Wash

Skin wash occurred following the final receptor fluid sample at 24 hours. The donor chamber was carefully removed and the underside (surface in contact with the membrane) wiped with one natural sponge pre-wetted with a dilute soap solution (3% Teepol L in water) which was added to the wash sponges. The donor chambers were washed with acetonitrile and the sample of the washing taken for analysis by LSC.

Sample Collection

Samples (0.1 mL for aqueous dilutions or 0.5 mL for the concentrate) of receptor fluid were taken from the receptor chambers of this static cell system 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application using an autosampler. The receptor fluid in the chambers was stirred continuously and the receptor volume was maintained by the replacement of a volume of fresh receptor fluid, equal to the sample volume, after each sample had been taken. The samples of receptor fluid were analysed by LSC.

After the final receptor fluid sample had been taken at the end of the exposure period, the remaining fluid in the receptor chamber was discarded. To remove residual receptor fluid from under the surface of the skin, the receptor chamber was again filled with fresh receptor fluid (approximately 5 mL) which was, afterwards, discarded.

The epidermal surface of the skin gently wiped with natural sponges pre-wetted with 3% Teepol L in water, and with further sponges pre-wetted with water. The sponges were digested in a solubilising agent (Soluene 350) and a sample taken for analysis by LSC.

For human tissue samples, successive layers of the stratum corneum were removed by the repeated application of adhesive tape (i.e. Scotch 3M Magic Tape, 1.9 cm wide), to a maximum of 5 strips. The skin surface was allowed to dry naturally. Strips of adhesive tape were sequentially pressed onto the skin surface and then carefully peeled off to remove layers of the stratum corneum. The adhesive strips were immersed individually in acetonitrile to extract any test material. The extracts were sequentially numbered and analysed by LSC. Tape stripping was discontinued if it was observed that the epidermis was beginning to tear. In such cases, the last strip taken was digested with the remaining skin to avoid underestimating residues in the remaining epidermis compartment.

Tape stripping cannot be performed with rat skin due to the fragility of the rat epidermis.

For all samples, the epidermis was removed from the receptor chamber, digested in Soluene 350 and the whole digest analysed by LSC.

Sample Preparation and Analysis

Information regarding sample storage before analysis was not provided.

All samples were analysed by Liquid Scintillation Counting (LSC). Where appropriate, a representative 'blank' sample was analysed to provide a background count that was deducted automatically from the particular batch of samples analysed. These background values were used to calculate the limit of quantitation (LOQ) using LSC, in each of the study compartments.

The LOQ for the receptor fluid was calculated using the pre-samples (background samples) taken from the receptor chamber of each cell and expressed as µg/cm^2 and % of applied dose. These pre-samples also provided background counts that were automatically deducted from the time course counts to provide meaningful data that were above the LOQ. As such, raw, uncorrected data was not provided for the samples from each diffusion cell.

The liquid scintillation counter automatically converted cpm to dpm with quench curve correction using an external standard.

For each dose level and species, the total mean amount of radioactivity in each sample was calculated and reported as a percentage of the total dose and as cumulative penetration (µg/cm^2). The relevant result with respect to occupational exposure assessment is dermal absorption represented as a percentage of the applied dose. This was determined by the combined total SYN545192 residues in the receptor solution and skin membrane (after skin wash) divided by the applied dose.

RESULTS

Summary Tables

See Table 6.12.2-2.

Total Absorbed Dose

Results of sample analysis were expressed as amounts of SYN545192 in µg/cm^2 as well as the percentage of dose. Results of analysis are summarised in Table 6.12.2-2. In the study report, values below the limit of quantitation (LOQ; 0.001µg/cm^2) were excluded from the calculation of group means. The PMRA assumed values below the LOQ to be equivalent to half of the reported LOQ; all other values were used as reported in the calculations of mean absorption and total recovery.

Estimates of dermal absorption were based on the sum of residues retained in skin membrane (after washing) + tape strips (for human tissue samples) + receptor fluid, which can alternatively be described as the sum of all residues except for skin wash and donor cell wash. This is consistent with the approach outlined in OECD (2011) because the amount recovered in the epidermis is potentially absorbable. Residues in all samples were added together determine the balance of radioactivity (recovery).

Results from one of the six cells were excluded from calculation of the mean (for the medium and high doses for rat skin and the high dose for human skin) by the study author since the cell showed significantly higher penetration values as compared to other cells. The applicant stated that absorption profiles indicated membrane damage during the course of the experiment. Since skin integrity for this cell was in the acceptable range and there did not appear to be reason to exclude these samples, PMRA included them in the calculation of the mean for these groups.

Mean recoveries for each group (dose level and species) ranged from 90.8 to 103%. All means fell within the acceptable range of 100 +/- 10% as determined by the OECD Guideline 428. Therefore, study results were not adjusted for incomplete recovery.

The mean total absorbed dose for human skin ranged from 0.55 to 3.17% of the administered dose. The mean was 0.55% for the high dose, 0.63% for the medium dose and 3.17% for the low dose. The mean total absorbed dose for rat skin ranged from 12.0 to 56.5 % of the administered dose. The mean was 12.0% for the high dose, 28.6% for the medium dose and 56.5% for the low dose.

In human skin, the majority of the administered dose was recovered from the skin wash (96 to 100%) for all dose levels. For rat skin, the majority was also found in the skin wash for the medium and high doses (73% and 84%, respectively) but for the low dose the majority was found in the receptor fluid (48%). The amount in the skin wash declined with decreasing dose levels, demonstrating the trend for increased dermal absorption at lower doses and saturation at higher doses. The results also showed that dermal absorption in rat skin is greater than in human skin.

DISCUSSION

LIMITATIONS OF THE STUDY:

As noted in the draft NAFTA Harmonization Position Paper on Methodology Issues (January 18, 1999), PMRA and U.S. EPA are in agreement that *in vitro* dermal absorption studies, alone, are not sufficiently validated for use in deriving estimates of systemic exposure for risk assessments. As such, the results of this study are of limited utility. The *in vitro* rat and human studies may be used together with an *in vivo* rat study in a triple pack approach, as discussed in a recent draft NAFTA position paper; however, this approach requires a number of parameters to be met (such as the dermal absorption between the rat *in vivo* and *in vitro* must be similar).

The following limitations were found and may impact the study results.

1. It appears that the pipette was not weighed before and after treatment; therefore, the actual amount of dose can not be determined. Similarly, raw data noting the actual amount of dose delivered was not available. If the full experimental dose was not delivered to the skin (i.e., some of the dose was retained in the pipette), this may underestimate the dermal absorption value.
2. Details regarding the components of the blank formulation were not provided. As per the OECD Guidance Notes on Dermal Absorption (2011), differences in formulation have the potential to affect the final dermal absorption percentage, thus, this creates an uncertainty regarding the acceptability of the study both independently and as a triple pack approach (since it also cannot be compared to the formulation in the rat in vivo study).
3. Skin Samples:
    a. Age of human skin donors. Samples were from elderly subjects (>69 years of age). Some studies have shown that barrier properties increase with age, while others have shown that permeability is relatively consistent throughout life after birth. Use of elderly subjects may result in an underestimate of dermal absorption for younger subjects (PMRA, 2009).
    b. No details were provided on whether or not the human skin donors had received any medical treatment or if the skin had been cleaned prior to removal. As some of these factors can influence the degree of dermal penetration, a lack of these details can limit the utility of the data.
    c. Upper leg samples from human cadavers were used but the OECD Guidance Document 28 indicates that abdominal or breast skin is normally used.
    d. For one sample, the source location of the human body was not available.
    e. Skin thickness was not reported for any of the tissue samples.
4. Some details on skin integrity were not provided, such as whether or not a physical check of the skin surface was conducted and the specific time period between the membrane integrity

testing and application of test material. Skin integrity may have deteriorated prior to the application of the test substance, as the guidelines indicate that skin integrity deteriorates after 24 hours. It also does not appear that membrane integrity tests were performed after application and sampling to compare integrity results before and after study.

5. Test System:
   Humidity was not reported. This is a limitation as it can effect dermal penetration. The guidelines specify that it should be between 30 and 70%.
6. The exposure time (application to wash) of 24 hr is not considered appropriate for typical exposure to agricultural chemicals. As noted in OECD Guidance Notes 156, typical exposure times would be from 6 to 10 hours before washing. The U.S. EPA Guidelines specify that exposure time is 10 hours. As such, the dermal absorption values from the studies may be conservative for the typical worker exposure duration.

CONCLUSIONS:

Dermal administration of low, medium and high doses of ^{14}C-SYN545192 (0.001, 0.01 and 1.5 mg ai/cm^2) was performed in an *in vitro* study using rat and human skin membranes.

Results of this study suggest that SYN545192 is readily absorbed through rat and human skin, and that dermal absorption is higher in rats than in humans. The mean dermal absorption in the rat skin test system was 12.0% for the high dose, 28.6% for the medium dose and 56.5% for the low dose. The mean total absorbed dose for human skin was 0.55% for the high dose, 0.63% for the medium dose and 3.17% for the low dose. The pattern of absorption also appears to be similar between rats and humans, with saturation at higher doses (i.e. greater dermal absorption at lower doses).

It is recognized that *in vitro* dermal absorption studies, alone, are not sufficiently validated for use in deriving estimates of systemic exposure for risk assessments (NAFTA, 1999). As such, the results of this study alone are of limited use. *In vitro* studies can be used by PMRA in a 'triple-pack' approach with an *in vivo* study (NAFTA, 2008).

EPA Conclusion: Correcting for tapes 1 & 2, the mean total absorbed dose for human skin was 0.47% for the high dose, 0.56% for the medium dose and 2.77% for the low dose.

Table 6.12.2-1 Dosing

Dose Level	Amount compound in dosing solution		Specific Activity (MBq)	Nominal Dose (mg/cm^2)	Actual Dose (mg/cm^2)
	Radiolabelled (µL)	Non-labelled(mg)			
concentrate	554.7	153.62	10.78	1.5	1.537
1/150	554.7	0	10.78	0.01	0.0101
1/1500	554.7	0	10.78	0.001	0.000986

Table 6.12.2-2: Recovery of SYN545192 in Rat and Human Skin at 24 hours Post-Application

Matrix	Mean SYN545192 Residues in Matrix (%)					
Species	Rat [1]			Human [1]		
Dose Level	Low	Medium[2]	High[2]	Low	Medium	High [2]
Dose (mg/cm^2)	**0.001**	**0.01**	**1.5**	**0.001**	**0.01**	**1.5**
Exposure	**24 h**	**24 h**	**24 h**	**24 h**	**24 h**	**24 h**
Donor Cell Wash	1.05 (0.95)	1.32 (0.83)	2.00 (0.31)	0.30 (0.29)	0.12 (0.28)	5.70 (3.76)
Skin Wash	33.28 (8.74)	70.05 (16.10)	83.93 (13.27)	99.95 (2.33)	96.40 (7.99)	100.87 (3.69)
Skin Membrane	8.29 (1.86)	8.91 (5.88)	5.40 (3.39)	0.51 (0.30)	0.11 (0.08)	0.13 (0.09)
Tape strips 1 & 2	NA	NA	NA	0.40 (0.18)	0.07 (0.05)	0.08 (0.05)
Tape strips 3-5	NA	NA	NA	0.24 (0.13)	0.03 (0.02)	0.06 (0.05)
Receptor Fluid	48.22 (12.80)	19.68 (3.56)	6.65 (4.04)	2.02 (0.87)	0.43 (0.25)	0.29 (0.43)
Total Recovery	90.84 (24.34)	99.96 (26.37)	97.97 (9.00)	103.41 (4.10)	97.15 (8.67)	107.11 (2.97)
Absorbed Dose (skin membrane + receptor fluid)	56.51 (14.65)	28.60 (9.45)	12.04 (7.20)	3.17 (1.48)	0.63 (0.40)	0.55 (0.59)
Absorbed Dose (skin membrane + receptor fluid) – Tape strips 1 & 2	56.51 (14.65)	28.60 (9.45)	12.04 (7.20)	2.77 (1.3)	0.56 (0.35)	0.47 (0.54)

[1] mean of 6 skin membrane replicates per dose level

[2] Results from one of the six cells were excluded from calculation of the mean by the study author since the cell showed significantly higher penetration values as compared to other cells. The applicant stated that absorption profiles indicated membrane damage during the course of the experiment. Since skin integrity for this cell was in the acceptable range and there did not appear to be reason to exclude these samples, PMRA included them in the calculation of the mean for these groups.

Table 6.12.2-6 References relied on

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N-R/NR	Owner
Annex II Data and Information					
	PMRA	2009	Dermal Absorption: Chemical specific adjustment factors and other uncertainty/variability in the evaluation of dermal absorption values. March 2, 2009.	NR	PMRA
	OECD	2004	Test Guideline 428: Skin Absorption: *in vitro* Method. Paris, Organisation for Economic Co-operation and Development.	NR	OECD
	OECD	2011	Guidance Notes on Dermal Absorption, Series on Testing and Assessment, No. 156. Paris, Organisation for Economic Co-operation and Development.	NR	OECD